Free Communications

Bone mineral density – Chronic kidney disease

**MO001** SUCCESSFUL INDUCTION OF TARGET GENES IN PARATHYROID CELL BY DIRECT INJECTION TECHNIQUE

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**Introduction and Aims:** We attempted to develop the gene induction in parathyroid cell (PTC) using with the established method of direct injection into hyperplastic parathyroid gland (PTG). The induction of target genes in PTC was investigated in uremia-induced advanced secondary hyperparathyroidism (SHPT), whose expressions of vitamin D receptor (VDR) and Ca-sensing receptor (CaSR) were significantly decreased.

**Methods:** 5/6-nephrectomized Sprague-Dawley rats were fed a high-phosphate diet for eight weeks. LacZ-induced adenovirus (LacZ-Ad) was administered by direct injection into PTG (DI), intravenously from jugular vein and direct application on the PTG surface. The PTCs were excised 48 hours after the treatment and were evaluated by X-Gal staining. Moreover, one of the PTCs was treated by DI of VDR- or CaSR-induced adenovirus (VDR-Ad and CaSR-Ad, respectively) and the other was treated by that of LacZ-Ad in the same rat. The immunohistochemical VDR and CaSR expressions of PTCs at the same time were investigated.

**Results:** Many X-gal-positive PTCs were observed in all over the PTG treated by DI of LacZ-Ad, however never in PTGs treated by the other administration of LacZ-Ad. Marked increases in the number of both VDR- and CaSR-positive PTG were also confirmed in PTG treated by DI of VDR-Ad and CaSR-Ad, respectively. The significant differences compared with individual the other PTG treated by DI of LacZ-Ad in the same rat were noted.

**Conclusions:** The induction of target gene in PTC of uremia-induced advanced SHPT model animal has been established. This technique may make it possible to investigate the various signaling pathways and changes of cell function and proliferation activity in PTCs inducted target genes.

**MO002** HIGH BONE AND CARDIOVASCULAR MORBIDITY IN FOLATE-RESISTANT HEMODIALYSIS PATIENT

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**Introduction and Aims:** Up to date meta-analysis of RCTs tell us that folate therapy might protect from cardiovascular events in patients with severe hyperhomocysteinemia responsive to folate therapy. Recent epidemiological studies affirm that hyperhomocysteinemia is associated with bone fragility. It might be necessary to normalize, as soon as possible, hyperhomocysteinemia for reducing the bone and cardiovascular events rate of dialysis patients.

**Results:** A few folate treated hemodialysis patients have normal homocysteine levels. Vitamin B12 therapy is necessary to correct cobalamin deficiency and to improve the homocysteine-lowering effect of folate therapy. It might be necessary to normalize, as soon as possible, hyperhomocysteinemia.

**Conclusions:** It might be necessary to normalize, as soon as possible, hyperhomocysteinemia.
and 446 patients had a second two-year follow-up LLR at the time of this analysis. Characteristics of the 446 patients included: M/F ratio was 64/36%, mean age 59 (19-87) years and duration of dialysis 39 (3-363) months; 18% of the pts were diabetics. 83% were treated with HD and 17% with PD.

At two years 377/446 (85%) had an AAC ≥ 1 vs. 346/446 (78%) at baseline. Calcification increased more in patient with calcification at baseline than in those without (ΔAAC 4.3±0.3 [mean±SE] vs. 2.5±0.5, respectively; P=0.0031). Among patients remaining on dialysis throughout the study 69/360 (19%) had AAC=0 at baseline of whom 31/69 (45%) patients had progressed (AAC≥1) at two years. Of patients transplanted during the study, 31/86 (36%) had AAC=0 at baseline. Six of these patients (19%) had calcification (AAC≥1) at two years. Progression of calcification was less pronounced in transplants vs. patients remaining on dialysis (ΔAAC 0.2±0.1 vs. 3.5±0.6, respectively; P=0.0143). 11/179 (6%) non-calcified (at baseline) patients died before study completion vs. 187/757 (25%) of those with AAC ≥ 1 (P<0.0001).

Conclusions: AAC progressed more in pts with calcification at baseline than in those without. Progression was much less pronounced in patients who received transplant during the study possibly partly due to positive selection bias. Baseline abdominal aortic calcification was a strong predictor of mortality. Lateral lumbar X-ray of the abdominal aorta may serve as a simple, low-cost prognostic indicator in dialysis patients.

Disclosure: Scientific advisor for Genzyme.

MO006  SODIUM BICARBONATE TO PREVENT ACUTE KIDNEY INJURY AFTER CARDIAC SURGERY: A PILOT DOUBLE-BLIND, RANDOMISED CONTROLLED TRIAL

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Introduction and Aims: To test whether perieroperative sodium bicarbonate infusion can attenuate acute kidney injury in cardiac surgical patients.

Methods: In a double-blind, randomised controlled trial we enrolled a cohort of 100 cardiac surgical patients at increased risk of postoperative acute kidney injury. Patients were randomised to either twenty-four hours of intravenous infusion of sodium bicarbonate (4 mmol/kg) or sodium chloride (4 mmol/kg). The primary outcome measure was the proportion of patients developing postoperative acute kidney injury defined as an increase in plasma creatinine concentration > 0.5 mg/dL (44 μmol/L) or > 25% of baseline within the first five postoperative days. Secondary outcomes included changes in plasma creatinine, plasma urea, urinary neutrophil gelatinase-associated lipocalin and urinary neutrophil gelatinase-associated lipocalin/urine creatinine ratio.

Results: Patients were well balanced for baseline characteristics. Sodium bicarbonate infusion increased plasma bicarbonate concentration (P<0.0001), base excess (P<0.001), plasma pH (P<0.0001) and urine pH (P<0.001). Fewer patients in the sodium bicarbonate group (16/50) developed postoperative acute kidney injury compared to control (26/50) (OR 0.43 [95% CI 0.19-0.98]), (P=0.043). The increase in plasma creatinine, plasma urea, urinary neutrophil gelatinase-associated lipocalin and urinary neutrophil gelatinase-associated lipocalin/urine creatinine ratio was less in sodium bicarbonate patients. (P=0.014; P=0.047; P=0.009; P=0.004). There were no significant side effects.

Conclusions: Sodium bicarbonate loading and continuous infusion was associated with a lower incidence of acute kidney injury in cardiac surgical patients undergoing cardiopulmonary bypass. The findings of this pilot study justify further investigation. (ClinicalTrials.gov, NCT00334191)
**MO007**

**ENDOTHELIAL PROGENITOR AND HEMATOPOIETIC STEM CELLS ARE SELECTIVELY RECRUITED TO THE KIDNEY AFTER INDUCTION OF SELECTIVE ENDOTHELIAL INJURY IN MICE**

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**Introduction and Aims:** Hematopoietic stem cells (HSC) and more specific precursor cells such as endothelial progenitor cells (EPC) are considered to contribute to the repair of tissue injury. However, few studies investigated this mechanism in the kidney, none after targeted microvascular endothelial injury. We therefore investigated the dynamics of HSCs, EPCs and cytokines after induction of a selective microvascular endothelial injury model in the mouse.

**Methods:** Selective, left-sided renal arterial perfusion of concanavalin A (con A) in mice by in vivo antibody was performed in 22 mice; 3 mice received PBS in lieu of con A and served as 1 hour controls. Mice were sacrificed 1h, 3h, and 5 days after induction of site-selective microvascular endothelial injury. CD150+/cKit+ HSC as well as CD34+/Flk-1+ EPC were isolated from peripheral blood, spleen and kidneys and analyzed by FACS. In parallel, various cytokines (including MMP-9, IL-6 + 10, TNF-α, G-CSF, GM-CSF, MIP-1α, KC, MCP-1, IP-10) were measured in serum samples. Tissue samples were obtained to verify disease induction and renal injury. Data was analyzed using one-way ANOVA. Single comparisons were performed using paired t-test.

**Results:** By histology, all kidneys showed signs of acute thrombotic microangiopathy. HSCs increased in the blood (over all, P < 0.05), spleen (3rd vs. 1hr, 3rd vs. control, P < 0.001) and left kidney (over all, P < 0.05). Direct comparison of left and right kidneys showed increased HSC content in left kidneys on day 5 (P < 0.01). The level of EPCs increased in left kidneys (P < 0.01), while it was unchanged in blood and spleen. On day 5, left kidneys retained more EPCs than contralateral kidneys of the same mice (P < 0.01). Early on (1-3 hours), MMP-9, MIP-1α, IL-6 and IL-10, TNF-α and KC levels were significantly elevated, whereas increased levels of GM-CSF, G-CSF and IP-10 were predominant after 3-5 days (all P < 0.05-0.001).

**Conclusions:** EPCs and HSCs are selectively recruited to the site of endothelial injury in this disease model, sparing the non-diseased right kidney. Increased numbers of circulating blood and splenic HSCs preceded their renal homing. Elevated levels of progenitor cell stimulating cytokines possibly play a role in recruitment of HSC and EPC after microvascular endothelial injury in the kidney.

**MO009**

**NGAL (NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN) AND CYSTATIN C COULD PREDICT DEVELOPMENT OF CONTRAST NEPHROPATHY AFTER PERCUTANEOUS CORONARY INTERVENTIONS IN DIABETIC PATIENTS WITH STABLE ANGINA AND NORMAL SERUM CREATININE**

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**Introduction and Aims:** The value of NGAL (neutrophil gelatinase-associated Lipocalin) and Cystatin C could be a sensitive biomarker of contrast nephropathy in patients with normal serum creatinine. In this study we tested the hypothesis whether NGAL could represent an early biomarker of contrast-induced nephropathy (CIN) in 70 diabetic patients with normal serum creatinine undergoing percutaneous coronary interventions-PCI in comparison to 70 sex- and age-matched non-diabetic patients. In addition, we assessed serum and urinary NGAL in relation to cystatin C, eGFR serum and urinary creatinine in these patients.

**Methods:** Serum, urinary NGAL (ANTIBODYSHOP, Gentofte, Denmark), cystatin C (Dade Behring, Germany) were evaluated before, and after 2, 4, 8, 24 and 48 hours after PCI using commercially available kits. Serum creatinine was assessed before, 24 and 48 hours after PCI, ANOVA or Kruskall-Wallis ANOVA for repeated measurements were used in statistical analysis.

**Results:** In both groups we found a significant rise in serum NGAL after 2, 4 and 8 hours, and in urinary NGAL after 4, 24 and 24 hours after PCI. At every time point serum and urinary NGAL were significantly higher in diabetic patients relative to non-diabetics. We found a significant rise in serum NGAL after 2, 4 and 8 hours (92.54 ± 31.87 ng/ml, p < 0.05, 120.59 ± 54.65 mg/ml, p < 0.001, 91.87 ± 41.76 mg/ml, p < 0.05 vs 78.43 ± 32.76 mg/ml at baseline), and in urinary NGAL after 4, 24 hours after PCI in non-diabetic patients. Serum NGAL was significantly higher in diabetics 2, 4, 8 and 24 hours after PCI (131.65 ± 87.65 mg/ml, p < 0.05, 143.65 ± 89.76 mg/ml, p < 0.001, 139.87 ± 84.54 mg/ml, p < 0.01, 129.59 ± 89.65 mg/ml, p < 0.05, vs 118.65 ± 68.54 mg/ml at baseline) as well as urinary NGAL which rose 4.8 and 24 hours after PCI. Serum cystatin C increased significantly 8 hours, reaching peak 24 hours.
after PCI in both groups and then decreased after 48 hours. Before PCI serum NGAL was related to creatinine, urinary NGAL, cystatin C in both groups. When contrast nephropathy was defined as an increase in serum creatinine by ≥25% of the baseline level 48 hours after PCI, the prevalence of CIN was 10% in non-diabetics and 14% in diabetics (all patients received low-osmolar contrast). Patients with CIN received significantly more contrast agent (p<0.01), but duration of PCI was similar. NGAL levels were significantly higher in patients with CIN starting 2 hours after PCI (serum NGAL) or 4 hours (urinary NGAL).

Conclusions: Diabetic patients are more vulnerable and prone to develop contrast nephropathy. Despite similar serum creatinine they seem to have more impaired kidney function. NGAL seems to be a potential early marker for nephrotoxicity and predictor of contrast nephropathy. It is particularly important in the upcoming setting of short-time hospitalizations for coronary angiographies and interventions.

**MO010 URINE NGAL PREDICTS SEVERITY OF ACUTE KIDNEY INJURY AFTER CARDIAC SURGERY: A PROSPECTIVE STUDY**

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**Introduction and Aims:** Cardiopulmonary bypass (CPB) surgery is the most frequent major surgical procedure performed worldwide. Acute kidney injury (AKI) after CPB is associated with several adverse outcomes, including prolonged hospital stay, dialysis dependency, and increased mortality. A major reason for the failure to find an effective treatment is the paucity of early biomarkers for AKI, and hence a delay in initiating therapies. Abbott Diagnostics has signed an exclusive licensing agreement and rapid diagnosis of AKI in the clinical setting, and will facilitate the investigation of promising therapeutic agents in humans with AKI.

The commercial platform for urinary NGAL determination will enable the early predictive biomarker of AKI severity after CPB. The availability of a standardized assay is easy to perform in any clinical laboratory, with no manual pretreatment steps, a result available within 35 minutes, and it requires only 150 microliters of urine. Urine NGAL is an early predictive biomarker of AKI severity after CPB. The availability of a standardized commercial platform for urinary NGAL determination will enable the early and rapid diagnosis of AKI in the clinical setting, and will facilitate the investigation of promising therapeutic agents in humans with AKI.

**Disclosure:** Abbott Diagnostics has signed an exclusive licensing agreement with Cincinnati Children’s Hospital and Columbia University for developing urine NGAL as a biomarker of acute renal failure.

**Results:** NGAL levels correlated with severity of AKI (r=0.66, p<0.001), duration of PCI (r=0.99). Second, in a subsequent validation study, 196 children undergoing CPB (NGAL range 0.3-815 ng/ml) and 6 calibration subjects undergoing CPB were prospectively enrolled, and serial urine NGAL measurements obtained by ARCHITECT assay. The primary outcome was AKI defined as a 50% or greater increase in serum creatinine.

**Results:** AKI developed in 99 patients (51%), but the diagnosis using serum creatinine increased 15-fold within 2 hours, and by 25-fold at 4 and 6 hours after CPB. For the 2 hour urine NGAL measurement, the area under the curve was the 0.95, sensitivity was 0.82, and the specificity was 0.90 for prediction of AKI using a cutoff value of 100 mg/ml. Within the AKI group, the 2 hour urine NGAL values were significantly higher in subjects who had either an earlier rise in serum creatinine or needed renal replacement therapy (both p<0.02). By multivariate analysis, the most powerful independent predictor of AKI was the early urine NGAL measurements (R²=0.68, p=0.0001). The 2 hour urine NGAL levels correlated with severity of AKI (r=0.86, p<0.001), duration of AKI (r=0.73, p<0.001), length of hospital stay (r=0.42, p<0.001), dialysis requirement (r=0.48, p<0.01), and mortality (r=0.53, p<0.01).

**Conclusions:** Accurate measurements of urine NGAL are obtained using the ARCHITECT platform. This assay is easy to perform in any clinical laboratory, with no manual pretreatment steps, a result available within 35 minutes, and it requires only 150 microliters of urine. Urine NGAL is an early predictive biomarker of AKI severity after CPB. The availability of a standardized commercial platform for urinary NGAL determination will enable the early and rapid diagnosis of AKI in the clinical setting, and will facilitate the investigation of promising therapeutic agents in humans with AKI.

**Disclosure:** Abbott Diagnostics has signed an exclusive licensing agreement with Cincinnati Children’s Hospital and Columbia University for developing urine NGAL as a biomarker of acute renal failure.

**MO011 ★ COMPARISON OF EIGHT-HOUR AND FOUR-HOUR THRICE WEEKLY HEMODIALYSIS**

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**Introduction and Aims:** Mortality rate in hemodialysis (HD) patients remains unacceptably high. Longer HD regimen seems promising in retrospective, uncontrolled studies. In this prospective, controlled study, we compared the effects of 8-h and 4-h thrice weekly HD.

Methods: We assigned 224 prevalent conventional HD patients to thrice weekly 8-h in-center nocturnal HD and 224 age-, sex-, diabetes status-, and HD vintage-matched control cases to 4-h HD for a year (Mean age 45±13 years, dialysis vintage 59±44 months, female 32%, diabetes 20%). Overall mortality (primary outcome), changes in clinical and laboratory parameters were evaluated. Cardiac structure and functions determined by echocardiography and quality of life, cognitive functions, and depression burden were assessed at baseline and 12th months.

**Results:** Mean follow-up was 12±5.0 months. Mean duration of HD sessions were 462±18 and 236±7 min, blood flow rates 241±47 and 291±35 ml/min in 8-h and 4-h arms, respectively. Overall mortality rates were 1.29 and 6.03 per 100 patient-year in 8-h and 4-h HD groups, respectively (p<0.01). Adjusted RR for death was 0.22 in 8-h group (95% CI 0.06-0.76; p<0.05). URR increased from 0.75±0.07 to 0.82±0.06, eKt/V from 1.48±0.34 to 2.62±0.89 in 8-h HD group (p<0.001). Post-dialysis body weight increased from 64.9±14.6 to 66.7±14.9 kg in 8-h group (p<0.001). Mean blood pressure and hemoglobin were similar in both groups during follow-up; use of anti-hypertensive medication and erythropoietin declined in 8-h group (from 24 to 8% and from 57 to 25%, respectively; p<0.01). Intradialytic hypotension episodes were significantly less in 8-h arm (19 and 85/1000 HD session, p<0.001). Albumin levels increased in 8-h HD group (from 3.95±0.29 to 4.10±0.30 g/dL, p<0.001). Despite reduction in use of phosphate-binder from 81 to 22% in 8-h arm, phosphate level decreased from 4.59±1.31 to 3.38±1.19 mg/dl (p<0.001); it rose from 4.82±1.27 to 5.03±1.12 mg/dl in 4-h group (p<0.001). 8-h HD group, left ventricular mass index regressed from 141±45 to 120±34 g/m² (p<0.01), left atrial diameter from 4.03±0.58 to 3.73±0.53 cm (p<0.001), left ventricular ejection fraction increased from 62±10 to 66±11% (p<0.001) in 8-h HD group. Cognitive functions, reflected by immediate and delayed recall scores, improved in 8-h HD group (p<0.05). Depression and anxiety scores did not change in both groups. Quality of life scores (mental health, vitality and bodily pain perceptions) deteriorated in 4-h HD group (p<0.05). If otherwise indicated no change was observed in group not mentioned.

**Conclusion:** These data point out that 8-h thrice weekly HD regimen provides a clear benefit on morbidity and mortality compared to conventional 4-h HD.

**Disclosure:** The study was supported by Fresenius Medical Care.
MO012 STENOSIS OF ARTERIOVENOUS FISTULA (AVF) IN REMOVALYSIS (HD) PATIENTS IS CHARACTERIZED BY REDUCED NEOANGIOGENESIS AND INCREASED FIBROSIS WITHIN THE ADVENTITIA

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Introduction and Aims: Vascular access failure is the main cause of morbidity in HD. Adventitial remodelling has been recently suggested to play a key pathogenic role in AVF stenosis, the major cause of vascular access failure. The aim of the present study is to evaluate adventitial neoangiogenesis and fibrosis in AVF stenosis and to elucidate the molecular mechanisms underlying these events.

Methods: We enrolled 44 patients undergoing surgery for AVF creation, 10 of them (mean age 52, range 33-72 years) presented AVF failure. During creation and revision of the vascular access, Fibrosis grade was evaluated by Masson's trichrome and quantified by a computerized image analysis system. The expression of CD34 (an endothelial cell marker), VEGF and its receptor Flk-1, alpha-smooth muscle actin (alpha-SMA), vimentin, total PDGF-beta receptor and its phosphorylated (activated) form were investigated by confocal microscopy.

Results: In native AVF, we observed a high expression of CD34 localized on the cell surface of endothelial cells lining the lumen of the vein and in the vasa vasorum within the vessel wall. In stenotic AVF, although we observed an increase in the venous wall thickness, CD34+ cells were significantly reduced, in particular within the adventitia (native AVF 3.6±3, stenotic AVF 0.6±2.0, p<0.01). As for CD34 also for Flk-1 we observed a striking and statistically significant reduction at the adventitial level of failed AVF (native AVF 13.1±3.8, stenotic AVF 7.8±1.6 p=0.009), while we did not observe any change in VEGF expression. Interestingly, there was a direct and significant correlation between CD34 and Flk-1 protein expression (r=41, p<0.05), suggesting a primary role for this receptor in the reduced angiogenesis within the adventitia. In this region, we observed a significant increase in deposition of extracellular matrix (native AVF 1.6±7, stenotic AVF 7.2±2, p<0.05). Adventitial fibrosis was characterized by a significant increase in a-SMA+ cells (native AVG 01±0.6, stenotic AVG 06±0.4, p=0.08). Most of these cells within the failed, but not native AVF were myofibroblast (a-SMA*Vimentin*). Interestingly, the extent of fibrosis was inversely correlated with Flk-1 protein expression (r=-57, p=0.02) and directly correlated with a-Sma expression (r=56, p=0.005). Finally, most of adventitial myofibroblast expressed high levels of PDGF-beta receptor. The role of this signaling molecule in the activation of these cells was confirmed by the observation of a marked up-regulation of its phosphorylation in the failed AVF.

Conclusions: Our data suggest that AVF failure is characterized by a reduction in adventitial angiogenesis and an increase in adventitial fibrosis potentially resulting in an abnormal vascular remodeling that, in turn, may promote AVF stenosis. In this context both VEGF and PDGF may play a key pathogenic role and represent potential therapeutic targets.

MO014 VALIDATION OF A BIOIMPEDANCE SPECTROSCOPY METHOD FOR THE ASSESSMENT OF FAT FREE MASS

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Introduction and Aims: The determination of body composition is an important aspect in many clinical fields. Even though simple measures like the body mass index (BMI) are widespread, it is often necessary to distinguish between active and passive body compartments. An everyday approach is to separate the total body weight (BW) into fat mass (FM) and fat free mass (FFM).

In a recent publication (AJCN 85, 2007) a three compartment (3C) body composition model was introduced expressing the body weight in terms of adipose and lean tissue mass (ATM, LTM), and excess fluid. It was the aim of this study to assess the validity of the 3C-model to determine FFM in healthy subjects and dialysis patients.

Methods: 146 subjects were analysed retrospectively, including 22 dialysis patients. Mean age of all healthy subjects was 44±18 yrs; BMI was 26.3±4.3 kg/m², covering a range between 17.5 and 40.9 kg/m². FFM_3C was determined by the 3C model using bioimpedance spectroscopy measurements (BCM-Body Composition Monitor, Fresenius Medical Care) of extracellular and intracellular fluid volumes as an input. FFM_3C was calculated with a 4C reference model (Fuller et al., Clin Sci, 1992) using BW, total body mineral content (TBMC), body volume (V) and total body water (TBW). TBMC was measured by dual energy x-ray absorptiometry (DXA); V was measured using air displacement plethysmography (ADP) and TBW was measured by deuterium dilution.

Methods: All rats received a peritoneal catheter implantation and a 70% nephrectomy (Nx). Thereafter the rats were randomly divided into 4 groups: GLAD (n=11), 3.86% Physioal (PG; n=5), 3.86% Dialysol (D; n=7) and Physiological without glucose (PF; n=13). All rats were infused daily for 16 weeks post Nx with the appropriate PDF. Thereafter a peritoneal permeability analysis (SPARA) was performed using 3.86% Physioal in all groups. Dextran 70 was used as volume marker. D/P creatinine, glucose absorption and fluid kinetics were calculated. Free water transport (FWT) at 60 min was calculated from transcapillary ultrafiltration and sodium transport. VEGF attributed to local production (LP) was calculated from the power relationship between D/P ratios of proteins and their molecular weights. Omental tissue, obtained after the SPARA, was stained with pico sirius red (PSR) for an overall fibrotic score (max 9) and with CD31 for vessel density. Total triglycerides were measured in plasma 1 hour after the infusions in week 14 in GLAD and PF.

Results: The groups could be compared, because both remnant kidney weight and renal creatinine clearance were similar in all groups. Results are shown in the table. Small solute transport and glucose absorption were significantly higher in all PDFs compared to PF. D had the highest scores for vessel density and fibrosis. Total triglycerides in plasma was not different between GLAD (1228±367 μmol/L) and PF (1351±170; ns).

| Parameter     | GLAD     | PG       | D         | PF       |
|---------------|----------|----------|-----------|----------|
| D/P creatine (mL/min) | 0.66±0.04 | 0.69±0.08 | 0.75±0.09 | 0.52±0.03 |
| Glucose absorption (%) | 71.0±1.3 | 71.0±3.7 | 72.0±0.8 | 62.6±1.5 |
| Total triglycerides (mg/dL) | 115±10 | 125±12 | 130±16 | 140±18 |
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| Total triglycerides (mg/dL) | 115±10 | 125±12 | 130±16 | 140±18 |

Conclusions: GLAD exposure was associated with the best preservation of peritoneal morphology and lowest LP-VEGF. The results of GLAD were very similar to those of PF containing the bicarbonate/lactate buffer, but without osmotic agents.

Disclosure: Part of this study was financially supported by grant of Baxter R&D Renal, Belgium.
Conclusions: FFM determined from the 3C model using BIS shows excellent agreement with a gold standard reference method. Since the 3C model also takes into account the excess fluid, it performs well not only in healthy subjects but also in patients with an abnormal hydration status.

Disclosure: Some of the authors are employed by the manufacturer of the bioimpedance device used in this study.

POLYSACCHARIDES ARE HYPERPERMEABLE ACROSS THE GLOMERULAR FILTRATION BARRIER, BUT NOT ACROSS THE CONTINUOUS PERITONEAL CAPILLARIES

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Introduction and Aims: Compared to neutral globular proteins, neutral polysaccharides, such as dextran, pullulan and Ficoll, appear hyperpermeable across the glomerular filtration barrier. This hyperpermeability has been attributed to an increased flexibility and/or asymmetry of polysaccharides vs. globular proteins. The aim of the present study was to investigate whether polysaccharides are also hyperpermeable, compared to globular proteins, across the continuous capillaries in the peritomeum in rats subjected to experimental peritoneal dialysis.

Methods: In anesthetized Wistar rats FITC-Ficoll or FITC-pullulan together with 125I-human serum albumin (RISA) were given intravenously as a bolus dose. Peritoneal dialysis using conventional peritoneal dialysis fluid (Gambroisol 1.36%) was performed for 120 min. Samples from plasma and dialysis fluid were analyzed using high performance size exclusion chromatography (HPSEC) for FITC-polysaccharides and a gamma counter and dialysis fluid were analyzed using high performance size exclusion chromatography (HPSEC) for FITC-polysaccharides and a gamma counter.

Results: Ficoll and pullulan showed more or less identical permeabilities, compared to globular proteins, across the peritoneal membrane. RISA-clearance 4.68±0.52 (μL/min; ±SEM) tended to be lower than the clearance of either Ficoll40K (5.16±0.06) or pullulan40K (5.3±0.50). This is in contrast to the hyperpermeability exhibited by polysaccharides across the glomerular filtration barrier.

Conclusions: It is speculated that the hyperpermeability of Ficoll and pullulan across the glomerular filter may be related to the very high degree of transport restriction exerted by the glomerular barrier, which is compatible with an equivalent small pore radius of only 37.4 Å. In peritoneal capillaries the apparent uncharged equivalent small pore radius is nearly twice that characteristic of the glomerular filtration barrier. The markedly lower size restriction offered by the continuous peritoneal capillary walls may explain why Ficoll40K or pullulan40K are much less affected by molecular flexibility in these capillaries. It is concluded that the phenomenon of molecular flexibility is more important for a macromolecule’s permeability through the glomerular filter than across the continuous peritoneal capillary endothelium.

MO016 SUCCESSFUL TREATMENT OF ANTI-ERYTHROPOIETIN ANTIBODY-MEDIATED PURE RED CELL APLASIA WITH HEMATIDETM

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Introduction and Aims: Pure red cell aplasia (PRCA) caused by anti-erythropoietin (EPO) antibodies is a rare, albeit serious complication in patients treated with protein-based erythropoiesis stimulating agents (ESAs), which has led to recommendations to limit their subcutaneous (SC) use. Treatment of anti-EPO antibody induced PRCA is currently unsatisfactory because of the side effects and limited success of immunosuppressive therapies. The aim of the present study was to test whether Hematidetm, a novel synthetic peptide-based EPO receptor agonist with no sequence homology to EPO, is able to stimulate erythropoiesis in patients with anti-EPO antibodies.

Methods: Ten patients with chronic kidney disease (CKD) who had proven anti-EPO antibody-mediated pure red cell aplasia or hypoplasia were enrolled in an open-label, prospective, non-randomised trial of Hematide administered by SC injection once every four weeks (Q4W). Haematological response, transfusion requirements, routine biochemistry, iron status, anti-EPO antibodies, anti-Hematide antibodies, and adverse events were monitored. The primary end-point was haemoglobin concentration above 11 g/dL in the absence of blood transfusions.

Results: After a median follow-up time of 13.5 months the median haemoglobin increased from 9.7 g/dL at baseline, with red cell transfusions in 9 of 10 patients, to 11.6 g/dL without transfusion support. Peak reticulocyte counts increased to 100–250x106/L, compared to a median of 21.3x106/L at baseline. No patient developed anti-Hematide antibodies, and anti-EPO antibodies declined over the course of the study in most patients. Three patients discontinued the study after receiving renal transplants. The other patients continue to be treated with Hematide and the haemoglobin has so far remained within the target range after up to 18 months of therapy. Hematidetm was well tolerated with no safety issues, and no Hematidetm-related serious adverse events were reported.

Conclusions: In CKD patients with anti-EPO antibody-mediated PRCA, Hematidetm abolishes the need for blood transfusions and maintains haemoglobin levels in the target range.

Disclosure: Consultancy honorarium from Affymax.

ANEMIA AND EPO RESISTANCE AS PROGNOSTIC FACTOR IN HEMODIALYSIS PATIENTS: RESULTS FROM THE RISCANVID STUDY

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Introduction and Aims: Cardiovascular (CV) and overall mortality in haemodialysis (HD) patients are associated with reduced level of haemoglobin (Hb) defined as failing to achieve the Hb target recommended by international guidelines K/DOQI (11-12 g/dL). In these pts EPO resistance is often associated with a state of chronic inflammation. The RISCANVID (Cardiovascular risk in dialysis) study is a prospective, observational study that includes all the haemodialysis patients afferent to all dialysis centres in the north-west part of Tuscany (1,235,062 inhabitants). Aim of the study was to elucidate the role of the various risk factors on mortality and morbidity in HD patients; in particular, the attention is here focused on the impact of anaemia and EPO resistance on CV and overall mortality.

Methods: Seven hundred forty-seven patients in HD (mean age 66±14.1

Anaemia
years, means dialytic age 69±76.8 months, diabetes 19%) were enrolled in the study at June 2004. Demographic, clinical and laboratory data of the whole population were registered as well as co-morbidity conditions and administrated drugs. EPO resistance index (ERI) was defined as the weekly EPO dosage (iu/kg/week)/Hb level (g/dL). C-reactive protein (high sensitivity assay nephelometric assay) and IL-6 (ELA, Bender) were centrally determined. During the 24 months follow-up overall mortality, cardiovascular (CV) mortality and CV major non fatal events were registered. Results: Our data confirm the high mortality rate in HD patients (overall mortality 12.9%/year; CV mortality 5.9%/year). 653 pts (76%) were receiving EPO (58%) or darbopoietin (18%). Pts with Hb level <11 g/dL (N= 223) showed an increased risk for CV mortality (RR 1.52, CI 0.98-2.34) and overall mortality (RR 1.58, CI 1.18-1.97) in comparison to pts with Hb levels 11-12 g/dL. On the basis of decile distribution of ERI pts hypo-responders; 10th decile: mean 31.46 iu/kg/week/g Hb, (N=63) showed an increased RR for CV (RR 1.7 CI 0.7-3.7, p<0.001) and overall mortality (RR 1.6, CI 1.2-2.6, p<0.001) considering as reference pts not receiving EPO (Hb 12.5±1.2 g/dL) (N=104). Furthermore, BMI and albumin were significantly reduced in the hyporesponders group in comparison to pts not receiving EPO (21.6±3.82 vs 24.3±3.72 kg/m², P<0.001; and 3.42±0.56 vs 3.78±0.34 g/dL, P< 0.001), whereas the mean CRP level was higher (16.5±5.3 vs 8.4±3.7 p<0.001 mg/l). Conclusions: Data at two years from the RISC/AVID study confirm anemia as an important risk factor for CV and overall mortality in dialysis patients. EPO responsiveness can also be considered a strong prognostic factor and seems tightly related to the malnutrition and inflammatory status of the uremic syndrome.

**MO018 EFFICACY AND SAFETY OF IV FERRIC CARBOXY-MALTOSE (FCM) COMPARED TO ORAL IRON IN ANEMIC PATIENTS WITHOUT NON-DIALYSIS-DEPENDENT CKD**

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**Introduction and Aims:** Iron sucrose is the only IV iron FDA approved as a first line treatment in non-dialysis-dependent CKD (ND-CKD) patients. The usual dosing regimen is 1000mg given in divided doses of 200mg during a 14 day period. This approach is often inconvenient in this population as it requires frequent clinic visits. It would be ideal to give 1000mg during a single clinic visit but concern about hypotension and other adverse drug events (ADEs) precludes this approach. In the present study, we compared the efficacy and safety of a new IV iron, ferric carboxymaltose (FCM) with oral FeSO4, in patients with ND-CKD.

**Methods:** This was a randomized, controlled multicenter trial. Inclusion criteria were GFR <45ml/min/1.73m², Hb <11g/dL, TSAT <25% and ferritin <300ng/ml. ESA doses, if any, were stable prior to and during the study. FCM was given as a maximum first dose of 1000mg over 15 minutes in patients enrolled in phase 3b identical studies (open-label, single-arm, multicenter), each with 250 patients were randomized; 147 patients allocated to FCM and 102 to FeSO4. The proportion of patients achieving the primary outcome (Hb increase > 1g/dl) was statistically significantly greater in the IV FCM group than in the FeSO4 group (60.4% vs. 34.7%; p<0.001), as were the % achieving 1g/dl increase in Hb by day 28, the mean change to highest Hb (1.3 vs 0.8g/dL), mean increase in Hb by day 42 (1 vs 0.5) and 56 (1 vs 0.7g/dL). Incidence of ADEs was 2.7% in the FCM group vs 26.2% in the FeSO4 group. No serious ADEs or hypotensive episodes were seen in patients administered FCM. Patients in the FeSO4 group but not the IV iron group showed a significant rise in serum creatinine at day 14 (0.07mg/dL, p=0.008) and 28 (0.07mg/dL, p=0.026)

**Conclusions:** In ND-CKD patients, 1000mg IV FCM over 15 minutes, with 1 to 2 additional 500mg doses, was convenient, more effective and associated with less ADEs than oral iron therapy.

**Disclosure:** This study was supported by a research grant from American Regent/Luitpold Pharmaceuticals (Shurely New York, US).
**Immune and inflammatory mechanisms**

**MO020**  
**SULODEXIDE – A HEPARINOID DRUG – INDUCES HEPATOCYTE GROWTH FACTOR RELEASE IN HUMANS**

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**Introduction and Aims:** Heparins influence numerous pleiotropic growth factors, including hepatocyte growth factor (HGF) – a multipotential regenerative cytokine - partially by their release from endothelial and extracellular matrix stores. The effects of sulodexide, a heparin-like glycosaminoglycan medication of growing importance in clinical nephrology (particularly in diabetic nephropathy primary glomerulopathies and atherosclerosis), on HGF liberation are not known.

**Methods:** We performed a 2-week open-label sulodexide trial in healthy male volunteers. The drug was initially administered intravenously (i.v.) in a single dose of 1200 Lipoprotein Releasing Units (LRLU), then - orally for 12 days (300 LRLU twice a day), and – again by i.v. route (1200 LRLU) on day 14.

**Results:** On day 1, HGF levels significantly changed following i.v. sulodexide administration (F = 854, P < 0.0001). They increased by 3557±802% - from 0.51±0.11 ng/ml at T0 to 18.2±2.62 ng/ml at T120 (P < 0.0001). At T120, HGF levels were 6.63±1.54 ng/ml lower than those at T100 (P < 0.0001) but still elevated by 1249±448% compared with the baseline values (P < 0.0001). Following oral sulodexide administration on day 7, plasma HGF values at T120 were not different from those at T0 (0.48±0.08 ng/ml vs 0.49±0.07 ng/ml, P = 0.740). On day 14, the i.v. sulodexide injection resulted in the striking and statistically similar increase in plasma HGF as that observed on day 1 (F = 786, P < 0.0001). The HGF levels were: T0 0.49±0.08 ng/ml, T10 17.8±1.66 ng/ml and T120 7.22±1.33 ng/ml; the percentage increments in plasma HGF were: T10 vs T0 3616±483% and T120 vs T0 1490±326%. The HGF values at the particular time points on day 14, as well as the magnitude of its increments were remarkably similar to those on day 1 (all P > 0.464). The T120 HGF levels measured on day 1, 7 and 14 did not differ from one another (F = 0.277, P = 0.761).

On day 1, the T120 HGF levels tended to be associated directly with i.v. sulodexide dose per kg of body weight (r = 0.598, P = 0.052). The relation between the percentage of HGF increase and this sulodexide dose was also remarkable and plausibly biologically and clinically important stimulating effect on the release of pleiotropic hepatocyte growth factor in humans.

**MO021**  
**SCREENING OF A KIDNEY cDNA LIBRARY FOR THE IDENTIFICATION OF AUTOIMMUNE PROTEINS FROM SERUM OF MEMBRANOUS NEPHROPATHY PATIENTS**

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**Introduction and Aims:** Membranous Nephropathy (MN) represents a large number of cases of Nephrotic Syndromes in the adult population and its distinctive diagnosis is currently carried out through biopsy. An autoimmune condition is suspected in MN in which some glomerular structures are targeted by patient antibodies. We do not know yet the target protein (or proteins) putatively involved and responsible for the disease. The aim of this work is to identify these proteins by screening a lambda- phage library using patient serum pools.

**Methods:** We set up the following three pools of sera: (i) from 15 MN patients, (ii) from 15 non-MN renal patients (4 with diabetic nephropathy, 4 with focal glomerulosclerosis, 4 with type 1 membranoproliferative glomerulonephritis, 3 with minimal change disease), and (iii) from 15 healthy individuals. A commercial cDNA phage library (from healthy kidney whole mRNA) was screened using the above described pooled sera, in order to detect positive signals following antigen-antibody recognition.

**Results:** We detected one phagelime clone expressing a protein which was shown to be targeted by the antibodies of the pooled MN sera only. Control sera were both negative. The cDNA insert carried by the phageclone was subsequently sequenced. In particular, by comparing the sequence we isolated with a human DNA database, a complete match with the synapomorph complex protein 65 (SC65), also known as NoS5, was found.

**Conclusions:** Anti-NoS5 autoantibodies could be involved in MN physiopathology as we observed through analysis of sera from affected patients. Considering the invasiveness and the risks associated with renal biopsies, little- or non-invasive methods such as blood sampling would be desirable. The identification of candidate patient autoantibodies targeting renal self proteins, might therefore help revealing new pathogenetic mechanisms of MN and, eventually, developing new diagnostic methods.
MO023  RENAL INFLAMMATORY INFILTRATE IN LUPUS NEPHRITIS PATIENTS

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Introduction and Aims: Pathogenesis and prognostic factors of lupus nephritis are still not completely known. Recent studies have revealed the implication of lymphocytic interstitial infiltrate in the pathogenesis and prognosis of SLE nephritis. We sought to evaluate the presence, distribution and phenotypes of the inflammatory infiltrate in renal tissue of SLE nephritis patients and possible correlations with clinical or histological parameters and response to therapy.

Methods: 31 consecutive patients with newly diagnosed lupus nephritis were included in the study. Demographic, clinical, immunological and renal evolution data were collected. Renal biopsies were classified according to the WHO criteria and examined for activity and chronicity indexes. In addition to routine staining, the tissues were stained by immunohistochemistry using as cellular markers CD3 (T lymphocytes), CD20 (B lymphocytes) and CD68 (macrophages). Cellular count was performed for each phenotype and results were expressed as number of positive cells on total biopsy surface. The infiltrate was considered as present if the number of total infiltrating lymphocytes was > 10 cells.

At 6th month of immunosuppressant therapy patients were considered responders or non responders on the basis of 24h proteinuria < 1 gr and creatinine levels reduction.

Results: Characteristics of the 31 patients were: 27 women, 4 men; age at renal biopsy 35.6 ± 11.6 years; number of class IV WHO = 31; activity index 9.1±4.3 and chronicity index 2.1±1.9. The number of CD3+, CD20+ and CD68+ cells were 17.7±23.4, 13.1±25.4 and 47.1±41.7, respectively. The leukocyte infiltrate was prevalently interstitial, without specific sublocalization. A direct correlation between chronicity index and presence of CD3 (r = 0.40, p = 0.025) and CD20 (r = 0.59, p = 0.001) positive cells was found. No correlations were observed with other clinical, laboratory or histological parameters. 13 out of 14 patients without infiltrate were responders with respect to 10/17 with infiltrate (93% vs 59%, p = 0.045). The baseline creatinine values (1.2±0.5 vs 1.0±0.7; p = 0.03) and the chronicity index (2.8±2.2 vs 1.2±1.0; p = 0.04) were higher in the second group. The 23 responder patients differ from the 8 non responders only for the presence of inflammatory infiltrate (43.5% vs 87.5%, p=0.045) and the chronicity index (2.1±1.9 vs 4.3±1.0; p = 0.001). The number of CD3+ cells (13.7±22.3 vs 29.4±24.0; p=0.03)

Conclusions: These data supported the hypothesis that the cell-mediated immunity play a central role in the SLE nephritis pathogenesis. The higher values of creatinine and chronicity index observed in patients with infiltrate suggest its possible function in the progression of the nephritis up to chronic damage. and which are the factors that are associated with this disturbance. The aim was to investigate possible changes of apoptosis and inflammation markers during the course of CKD progression and see whether there is any association between them.

Methods: In a cross-sectional study we studied 152 steady patients from the out-patient clinics of two Hospitals. Their mean age was 62 years old (range 28-88 years) and there were 83 males (55%). The most common primary renal diseases were hypertensive nephrosclerosis (15%), diabetes (12%) and glomerulonephritis (11%). Apoptosis was assessed in PBMC (lymphocytes and monocytes) at recruitment of these patients by estimating Bcl-2 expression using flow cytometry, Annexin V and Propidium Iodid as a marker for the detection of early apoptotic cells and plasma Fas and Fas-ligand activity. At the same time we estimated the levels of plasma CRP, TNFα and IL-6 as markers of inflammation.

Results: Bcl-2 expression was found to decrease significantly both in lymphocytes and monocytes as CKD stages were advancing suggesting that the antiapoptotic activity declined significantly with the progression of CKD. In contrast the activity of the apoptotic Fas increased significantly and so did plasma levels of TNFα from CKD stage 1 to 4. Results are as shown in the table below and values are expressed as median±SE. The distribution of patients to CKD stages and their eGFR (MDRD) are also shown below (mean±SD) (table).

| CKD 1 | CKD 2 | CKD 3 | CKD 4 | ANOVA, p |
|-------|-------|-------|-------|-----------|
| eGFR ml/min | 109±16 | 75±8 | 43±8 | 23±4 | <0.001 |
| Bcl-2 Lymphocytes |
| molecules/cell | 1801±115 | 1799±97 | 1508±59 | 1356±81 | <0.001 |
| Bcl-2 Monocytes |
| molecules/cell | 833±49 | 831±51 | 726±48 | 657±30 | <0.04 |
| Fas pg/ml | 9176±746 | 8542±369 | 1423±461 | 1346±246 | <0.001 |
| TNFα pg/ml | 1490±14 | 2224±29 | 237±10 | 297±0.22 | <0.003 |

Annexin V, Fas-ligand, IL-6 and CRP did not change with the progression of CKD. An interesting interleukin between apoptosis and inflammation markers was found. Although CRP did not increase with CKD stages, it showed a significant correlation with Bcl-2 Lmedian (p<0.01), with Annexin V LMEA (p<0.05) and IL-6 (p<0.001). IL-6 was correlated negatively with bcl-2 Lmedian (p<0.05) and positively with Fas (p<0.05) and TNFα (p<0.001). Finally, TNFα correlated significantly with Fas (p<0.001).

Conclusions: It appeared that apoptosis increased as CKD progressed to end stage renal failure and this was associated with increased pro-inflammatory activity. This finding deserves further investigation in order to clarify the complex association of apoptosis and inflammation during the early stages of CKD.

MO024 ★ APOTOPSIS IN PERIPHERAL BLOOD AND INFLAMMATION MARKERS IN PATIENTS WITH CHRONIC KIDNEY DISEASE (CKD)

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Introduction and Aims: Increased apoptosis of circulating blood leucocytes has been described in haemodialysis and non dialysis patients. However, there is limited information at which stage during the progression of CKD the balance between pro-and anti-apoptotic mechanisms is disturbed

Inflammation and wasting

MO025 PERIODONTAL DISEASE, INFLAMMATION AND NUTRITIONAL STATUS IN IN HAEMODIALYSIS PATIENTS

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Introduction and Aims: Chronic infection and inflammation, as well as malnutrition have been associated to an increased risk for atherosclerosis and therefore a poorer survival in haemodialysis (HD) patients. Recent studies revealed dental infection, especially periodontitis, to be associated with inflammation, malnutrition and increased risk for atherosclerosis. We therefore aimed to assess the possible relationship among periodontal disease, inflammation and malnutrition in HD patients.

Methods: All the stable HD patients from a single center, without malignancies or acute illness were evaluated in a cross-sectional single-center observational study. Periodontal status was assessed by gingival index (GI), papillary bleeding index (PBI), plaque index (PI), loss of clinical attachment level (CAL), according to the WHO recommendations, by a single examiner. Patients were stratified using the periodontal disease score: healthy dental status (PDS 0-2), mild periodontitis (3-4), moderate periodontitis (4-5), and severe periodontitis (5-6). Demographic, smoking status, hematology, dialysis-related data and parameters of the nutritional status...
SERUM SOLUBLE VEGFR-1 IN PREVALENT CHRONIC KIDNEY DISEASE (CKD) AND MUSCLE MASS DEPLETION AND WEIGHT LOSS

Methods: However, to date no one has evaluated sVEGFR-1 in CKD. Previous studies have shown that VEGFR-1 is elevated in the circulation of patients with cardiovascular disease and wound repair. However, to date no one has evaluated sVEGFR-1 in end-stage renal disease (ESRD). The aim of the present study was thus to evaluate circulating sVEGFR-1 in ESRD, and to relate it to inflammation, protein-energy wasting, cardiovascular disease as well as survival.

Results: Two hundred and nineteen HD patients (age: 54.5±12.8 years; 57% males; HD vintage 9.3±6.9 yrs; ESRD caused by primary glomerular nephropathies in 48% cases, with 3% diabetic nephropathy) were evaluated. Twelve patients (5%) were excluded of edentulism or absence of at least two teeth within any of the sextants. Poor oral health status was described in 84% of the evaluated hemodialysis patients. 22% of them had mild, 50% moderate and 28% severe periodontitis. Periodontal disease was significantly more frequent in patients older than 65 (97 versus 80%), in smokers (90 versus 67%) and in diabetics (100 versus 83%). Patients with periodontitis had longer HD duration (10.2±3.8 versus 6.4±1.8 years).

Periodontal disease was more prevalent in patients with malnutrition (SGA B, 97 versus 80%) and in those with inflammation (CRP> 5 mg/L, 91 versus 78%). In patients treated with erythropoietin-stimulating agents, erythropoietin resistance index was significantly higher in patients with moderate and severe periodontitis than in those with healthy dental status (median [interquartile range]: 10.3 [9.2, 15.7] versus 6.9 [2.9; 8.7] IU/kg per week per 1 g/dL haemoglobin).

Conclusions: Periodontal health is highly prevalent in hemodialysis patients and is more frequent in elderly, in diabetics, in smokers and in those with longer HD vintage. The prevalence seems to be higher in patients with malnutrition and in those with inflammation and to influence anaemia response to the treatment and, therefore, the outcome.

INTRODUCTION AND AIMS: Soluble vascular endothelial growth factor receptor (sVEGFR)-1 is a soluble form of the VEGFR-1 that may function as a VEGF-A inhibitor. Previous studies have shown that VEGFR-1 is elevated in many disease states, and related to inflammation, cardiovascular disease and wound repair. However, to date no one has evaluated sVEGFR-1 in end-stage renal disease (ESRD). The aim of the present study was thus to evaluate circulating sVEGFR-1 in ESRD, and to relate it to inflammation, protein-energy wasting, cardiovascular disease as well as survival.

Methods: sVEGFR-1 was measured in 202 prevalent hemodialysis patients (56% male, age 63±14 years) and 51 age-matched control, using commercial ELISA. Levels were related to clinical characteristics, biochemical markers, and survival. The patients were followed prospectively for a median 31 months (range 3–42 months).

Results: Serum sVEGFR-1 was significantly higher in patients than in controls (median 157.30 [87.60–382.40] vs 75.10 [56.94–107.54] pg/ml, p<0.001). Patients with overt muscle atrophy and/or loss of subcutaneous fat had higher sVEGFR-1 levels than those without these changes. In univariate analysis, sVEGFR-1 levels were positively correlated with IL-6 (rho=0.226, p=0.001), CRP (rho=0.136, p=0.054), white blood cell count (rho=0.234, p=0.001), and negatively correlated with serum albumin (rho=-0.194, p<0.001) and handgrip strength (rho=-0.255, p<0.001). In multivariate analysis, sVEGFR-1 was independently associated with muscular atrophy (Chi-Square=7.72, p=0.0055), after adjustment for inflammation, gender, age, and Davies score. In survival analysis, after adjustments for age, gender, vintage, Davies score and IL-6, patients with a sVEGFR-1 >131.2 pg/ml (a value determined by the receiver operating characteristics curve to predict outcome) fared significantly worse than those with a level below this value (HR=1.839, p=0.0148).

Conclusions: We conclude that sVEGFR-1 is a novel independent risk factor for mortality in prevalent hemodialysis patients, and that the levels of this marker are related to signs of protein-energy wasting and inflammation.

Disclosure: Bengt Lindholm is employed by Baxter.

Disclosure: Renée de Mutsert 1, Diana Groothondorf 1, Elisabeth Boeschoten 2, Raymond Krediet 1, Saskia Le Cessie 3, Friedo Dekker 1, Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, Netherlands; 2Hans Mak Institute, Nuenen, Netherlands; 3Department of Nephrology, Academic Medical Center, Amsterdam, Netherlands; 4Department of Medical Statistics and Bio Informatics, Leiden University Medical Center, Leiden, Netherlands

Introduction and Aims: A low body mass index (BMI) has been associated with increased mortality in hemodialysis patients. This study investigated whether this association between BMI and mortality is due to low fat mass or low muscle mass and whether recent weight loss may explain the high mortality risk associated with low BMI.

Methods: In a prospective observational cohort study end-stage renal disease patients of 18 years who started with their first renal replacement therapy were included in 38 dialysis centres in The Netherlands. Research nurses assessed BMI and skinfold thickness at three and six months after the start of dialysis and subsequently at 6-month intervals during 2.5 years of follow-up. Arm muscle area was calculated as an estimate of muscle mass and the sum of 4 skinfolds was calculated to estimate fat mass. With Cox regression analysis, relative mortality risks (HR) were calculated for baseline and time-dependent anthropometry, and for 6-month changes in anthropometry. All analyses were first adjusted for age, sex, smoking, and additionally for primary kidney disease and comorbidity.

Results: In total, 958 hemodialysis patients were included (age: 63±14 years, BMI: 24.6±4.2 kg/m²). During follow-up, 253 patients died, 128 as a result of CVD. BMI<18.5 kg/m² was associated with an increased mortality risk, both at baseline (HR: 2.00, 95%- CI: 1.08-3.71) and time-dependently (2.21, 1.25-3.91). Independent from BMI, muscle mass depletion at baseline was associated with an increased mortality risk (1.54, 1.07-2.23), as well as time-dependently (1.63, 1.12-2.38), whereas fat mass was not associated with mortality. Time-dependent weight change only explained a minor part of the risk of a low BMI (decreased to 1.81, 0.93-3.51). In the same analysis, it was apparent that time-dependent weight loss of 1-5% (HR: 1.52, 95%-CI: 1.06-2.16) and >5% (HR: 2.18, 95%-CI: 1.44-3.29) remained associated with increased risks of all-cause mortality, independent of comorbidity and the level of BMI.

Conclusions: Both muscle mass depletion and weight loss were associated with increased mortality independent of the level of BMI. This implies that obesity may not protect against the hazardous effect of wasting.

Unintentional weight loss during time on hemodialysis should be a warning signal independent of the BMI of the patient.

Disclosure: Giovanni Pertosa 1, Silvia Porreca 1, Giuseppe Dallino 1, Simona Simone 1, Marco Ciccone 1, Carmen Cosola 1, Stefania Pietanza 1, Cosima Balestra 1, Carlo Munno 1, Francesco Paolo Schena 1, Giuseppe Grandaliano 1.

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Introduction and Aims: CKD is a risk factor for cardiovascular disease (CVD) with decline in glomerular filtration rate (GFR) associated with a greater cardiovascular mortality. Vascular calcification contributes to accelerated atherosclerosis in CKD patients (pts). The process of vascular calcification appears to involve a phenotypic change in vascular smooth muscle cell, induced by BMP-2 and inhibited by BMP-7, resulting in a cell-mediated mineralization of extracellular matrix. The role of CKD and chronic inflammation in the modulation of BMP’s expression is unknown.

MO028
CHRONIC KIDNEY DISEASE (CKD) AND CARDIOVASCULAR RISK: IS THERE A ROLE FOR BONE MORPHOGENETIC PROTEINS (BMP)?

Biomarkers and progression
The aim of the present study was to evaluate BMP 2 and 7 serum concentrations in CKD pts (n=93, mean age 56.3 years (range 18-72)) and to investigate their relationship with GFR, high sensitive C reactive protein (hsCRP), intact (i) PTH serum concentrations and carotid Intima Media Thickness (cIMT), as a marker of atherosclerosis.

**Methods:** BMP 2 and 7 serum levels were measured by ELISA (R&D; RayBiotech, respectively), high sensitive C-reactive protein (hsCRP) by immunonephelometry and cIMT by B-mode ultrasonography. Estimated (e) GFR was assessed by MDRD formula. Study population was spread evenly for age and sex in K-DOQI stages.

**Results:** In our population eGFR was inversely correlated with cIMT (r<0.4; p<0.04). CKD pts presented significantly higher BMP-2 levels than normal subjects (C) (C 13.3±1.8; CKD 34.4±2.8 mg/ml, p<0.0001). On the contrary, lower BMP-7 serum levels were observed in CKD pts compared to C (C 274.4±30.3, CKD 69.7±4.8 mg/ml, p<.0001). Pts with stage 3-5 CKD presented higher BMP-2 (48.6±2.5; p<0.0001) and lower BMP-7 serum levels (14.8±1.7; p=0.0001) than stage 1-2 pts (BMP-2 23.8±2; BMP-7 64.4±3.9). BMP-2 directly correlated with eGFR (r=0.4; p<0.009) and hsCRP (r=0.4; p<0.05); whereas, BMP-7 was inversely correlated with eGFR (r=-0.4, p=0.04), hsCRP (r=-0.5; p<0.01) and iPTh (r=-0.6; p=0.02). cIMT significantly correlated with BMP-2 (r=0.4; p=0.01) and BMP-7 (r=0.6; p<0.04). In a multiple regression model, eGFR, hsCRP and BMP-2 all independently correlated with cIMT.

**Conclusions:** In conclusion, our data confirm that eGFR reduction may increase the risk for CVD and suggest that BMP-2 and BMP7 production, modulated by renal function and inflammation, may significantly influence the atherosclerotic risk in CKD.

**References**

1. Cantor, Thomas L., Yang, Z., Hughes, P. (2008). Biomarkers and progression. ii221

**Disclosure:**

Thomas L. Cantor is the founder/owner of Scantibodies Laboratory, Inc., Santee, CA, USA.

**Introduction and Aims:** Plasma from 10,000 ESRD patient specimens was pooled to produce 3 levels of PTH corresponding to below the K/DOQI guidelines for optimum patient outcomes. Differences in second generation intact PTH assays demonstrates K/DOQI guidelines inappropriate for use with several assays.

**Methods:** The 2006 survey was completed with distribution to over 400 clinical testing laboratories within Europe, U.S.A. and Asia. The K/DOQI guidelines were based on 73 articles that all referenced the Nichols Allegro IRMA iPTH assay. Those guidelines are appropriate for use only with the Nichols IRMA or an iPTH assay that is aligned with the Nichols Allegro IRMA iPTH assay. The Scantibodies total PTH IRMA assay was demonstrated and published as aligned with the former Nichols IRMA. Therefore, the Scantibodies iPTH assay was used as the reference assay to which other iPTH assay were compared.

**Results:**

- Several iPTH assays (including the Bayer Centaur, DPC Immulite and Abbott Architect) were found to be inappropriate for use with the K/DOQI guidelines because of overestimation of PTH. Several assays (including DiaSorin’s IRMA iPTH) were found to be inappropriate for use with K/DOQI because of underestimation.

- Overestimation or underestimation of PTH can lead to inappropriate treatments, so assays must be chosen carefully for use with the K/DOQI guidelines for optimum patient outcomes.

- Four main questions were addressed in this study:
  1. **Accuracy:** How accurate is the assay?
  2. **Linearity:** How well does the assay measure low values?
  3. **Precision:** How precise is the assay?
  4. **Range:** How does the assay measure high values?

- Methods:
  - The specimens were lyophilized and subjected to accelerated 10 specimens that included specimens containing synthetic 1-84 PTH and 150-300 pg/m/ml target, within the 150-300 pg/m/ml target and above the 150-300 pg/m/ml target.
  - The specimens were pooled to produce 3 levels of PTH corresponding to below the K/DOQI guidelines for optimum patient outcomes.
  - A good agreement was found between predicted GFR and measured GFR:
    - a. A very high logarithmic correlation was found between Cys and GFR. The correlation coefficients r were 0.91 for Cys Neph and 0.93 for Cys Turb.
    - b. The interassay coefficients of variation for Cys Turb were 0.729±0.205 mg/L in males, 0.714±0.233 mg/L in females (NS). The interassay coefficients of variation for Cys Turb were 2.68% and 1.81%, using two different pool of serum with a mean Cys concentration of 1.14 and 2.44 mg/L respectively.

- A very high logarithmic correlation was found between Cys and GFR. The correlation coefficients r were 0.91 for Cys Neph and 0.93 for Cys Turb.

- Formulas to predict GFR n the basis of Cys, developed on the basis of the relationship with GFR, were:
  - GFR = 73.084 x (Cys Neph) -1.0642; GFR = 67.752 x (Cys Turb) -1.2324

**Figure 1**

A good agreement was found between predicted GFR and measured GFR: the mean differences were 0.5 ml/min/1.73 m² using Cys Neph and 0.3 ml/min/1.73 m² using Cys Turb. The range of agreement resulted smaller using Cys Turb than Cys Neph.

**Conclusions:**

- The new immune-turbidimetric method proposed to measure serum concentration of cystatin C has a very low analytical variability and
seems adequate, at least like the classical immune-nephelometric method, to predict GFR and its impairment in CKD.

**MO031 ENDOTHELIAL PROGENITOR CELL NUMBER IN PERIPHERAL BLOOD AND PROGRESSION OF CHRONIC KIDNEY DISEASE**

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**Introduction and Aims:** Chronic kidney disease (CKD) and end stage renal disease are epidemic worldwide. The detrimental factors of renal function namely: high blood pressure, metabolic derangement such as hyperglycemia, dyslipidemia, oxidative stress state damage the microvasculature of kidney which lining by endothelial cells. The renal vascular histopathology of CKD included glomerulosclerosis, vascularopathy, and decrease of peritubular capillaries. Rationally, the progression of CKD depends on the balance between the detrimental factors and renal function restoration factors such as the regenerative process by endothelial progenitor cells (EPCs). EPCs are derived from bone marrow stem cells and behave as a reparative cell for vascular neo- genesis and restoration of endothelial function. We hypothesize that EPCs number correlate to CKD state and the deterioration rate of renal function.

**Methods:** We analyzed EPC numbers in peripheral blood of 60 CKD patients (20 of CKD stage II, 15 of CKD stage III, 15 of CKD stage IV and 10 of pre-dialysis CKD stage V) by using FACS analysis of VEGFR-2 and CD133 phenotype marker. The PBMCs were separated from ACD blood by Ficoll density-gradient centrifugation. The dump channel namely: T cell by CD3 marker, B cell by CD19 marker and myeloid cell by CD33 marker were excluded by monoclonal antibody to CD3, CD19 and CD33. EPC by cell culture assay was also analyzed by cell culture method using ac-LDL and UEA immunofluorescence staining and count by microscope. The GFR was measured by abbreviated MDRD formula.

**Results:** There were correlation of EPCs number by flow cytometry and cell culture assay (R2 = 0.765; p = 0.015). There were significant differences of EPCs numbers in peripheral blood by flow cytometry among CKD states (p = 0.014). The earlier CKD stage had higher number of EPCs (1,695±423 cells/mL for stage II, 1,234±312 cells/mL for stage III, 1,002±228 cells/mL for stage IV and 654±107 cells/mL for stage V). EPC numbers, both by flow cytometry and cell culture assay had correlation with GFR (R2 = 0.698; p = 0.013 by flow cytometry and R2 = 0.572; p = 0.01 by cell culture assay). Among 60 CKD patients, there were 47 patients who had deterioration rate of GFR more than 5 mL/min/1.73 m2/year (mean of 8.8±3.5 mL/min/1.73 m2/year) and 13 patients who had deterioration rate of GFR less than 5 mL/min/1.73 m2/year (mean of 0.4±0.2 mL/min/1.73 m2/year). EPC numbers by flow cytometry in patients with stable GFR (GFR decreased less than 5 mL/min/1.73 m2/year) were higher than patients who had deteriorating GFR (1475±548 cells/mL vs. 994±346 cells/mL; p = 0.014).

**Conclusions:** EPCs number measurement using VEGFR-2, CD133 phenotype staining marker by flow cytometry correlates with cell culture assay using ac-LDL and UEA immunofluorescence staining. The more advance stage of CKD had lower EPCs numbers in the peripheral blood. CKD patients who had deterioration rate of GFR had lower EPCs number in the peripheral blood compared with CKD patients who had stable GFR.

**MO032 ASTRAGALUS MONGHOLICUS BLOCKS TUBULAR EPITHELIAL TO MYOFIBROBLAST TRANSITION BY INDUCING THE EXPRESSION OF HEPATOCYTE GROWTH FACTOR AND ITS RECEPTOR C-MET IN RATS WITH UNILATERAL URETERAL OBSTRUCTION**

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**Introduction and Aims:** Although some studies have suggested that Astragalus mongholicus (AM) have beneficial effects on renal disease, its effects on renal tubulointerstitial fibrosis have not been completely clarified. The aim of this study was to investigate the effect of Astragalus mongholicus on the expression of hepatocyte growth factor (HGF) and its receptor C-met in Sprague-Dawley rats with Unilateral Ureteral Obstruction (UUO) and to elucidate the mechanisms underlying the renoprotective effects of Astragalus mongholicus.

**Methods:** 96 Sprague-Dawley rats were randomly divided into 4 groups: sham-operation group (Sham),the UUO group (UUO), UUO + AM group (AM) and UUO + Losartan group (ARB). One hour after UUO operation, ARB groups were intragastric administrated with losartan 20mg kg-1·d-1, while Sham, UUO and AM groups were intragastric administrated with identical voluminal normal saline. AM groups were intraperitoneal injected with astragalas mongholicus 10g kg-1·d-1, while Sham, UUO and ARB groups were intraperitoneal injected with identical voluminal normal saline. At 3, 7, 14, 21 days after UUO, the dynamic histological changes of renal interstitial tissues were observed and renal damage including tubular lesions and interstitial fibrosis were quantified on HE and Masson stained tissue sections. The expression of HGF, C-met, transforming growth factor-β1(TGF-β1) and α-smooth muscle actin (α-SMA), a marker of tubular epithelial to myofibroblast transition, were measured by immunohistochemistry staining sections. The mRNA of HGF, C-met, TGF-β1 and α-SMA were reversely transcribed and quantified by real-time PCR. HGF, C-met, TGF-β1 and α-SMA protein were detected by Western blot.

**Results:** Renal lesion was exacerbated and the expression of TGF-β1 and α-SMA were significantly increased in UUO group compared with those of Sham group (P<0.05) at each time point, while the expression of HGF and C-met were significantly increased at 3rd and 7th day (P<0.05). The expression of HGF and its receptor C-met increased at 3rd day and peaked at the 7th day after UUO and then decreased greatly at 14th and 21st day, while the expression of TGF-β1 peaked at 14th day. After AM intervention, tubular impairment and interstitial fibrosis were alleviated, up-regulations of expressions of TGF-β1 and α-SMA were significantly suppressed, while expression of HGF and C-met were significantly increased compared with UUO group (P<0.05) at each time point at nucleic acid and protein level. And there were no difference between AM and ARB groups (P>0.05).

**Conclusions:** Our results revealed that Astragalus mongholicus could induce the expression HGF and its receptor C-met and inhibit TGF-β1 expression, and therefore suppress the transdifferentiation of tubulo-epithelial mesenchyme. Our study suggested one possible mechanism by which Astragalus mongholicus contributed to retard the progression of renal tubulointerstitial fibrosis in UUO rats.
**MO033**  
**CONGENITAL ANOMALIES OF THE KIDNEY AND URINARY TRACT: EVALUATION OF THE LONG TERM OUTCOME BASED ON A SINGLE CENTRE EXPERIENCE**

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**Introduction and Aims:** Congenital Abnormalities of the Kidney and of the Urinary Tract (CUTAKUT) represent a major cause of chronic renal failure at any age. Data regarding prevalence and prognosis of CUTAKUT still remain elusive.

We report a single centre experience on 312 patients with various renal and urinary tract anomalies which have been followed for 5-30 years. This represents a complete and easy to use database for estimating the renal survival in patients with renal/urinary tract malformations, owing to the relevant number of patients, the homogeneity of the clinical approach and the long term follow-up.

**Methods:** The following categories were enrolled: A) solitary kidney with or without defects of the urinary tract (n 71); B) unilateral renal hypoplasia with/without urinary tract defects (n 93); C) bilateral renal hypoplasia with/without urinary tract defects (n 19); D) renal hypoplasia associated with stenosis of urethral valves (n 68); E) multicystic kidney (n 40); F) horseshoe kidney (n 21). Data were collected on demographics, type of malformation, date at diagnosis, imaging studies, specific surgical treatment and date of onset of end stage renal disease. Survival analysis was estimated according to Kaplan Meyer. Multivariate analysis was performed by Cox regression model.

**Results:** A significantly different clinical course was observed between the subgroup affected by vesicoureteral reflux (n=19) had a remarkably worse prognosis (ORS=0% vs. 100%) and the other three patients recovered enough renal function to be off treatment. Within category A the subgroup affected by vesicoureteral reflux (n=19) had a remarkably worse prognosis (ORS=0% vs. 42.3% at 30 yrs, p=0.07).

**Conclusions:** Our results clearly demonstrated different outcomes in terms of renal survival among different group that were only partially expected on the basis of the literature. Patients with horseshoe kidney didn’t require replacement therapy after a follow up of 20 years. Moreover, patients with renal hypoplasia and with solitary kidney showed an intermediate prognosis that was however relevantly poor, suggesting the existence of subclinical defects of the unique kidney. This represents a new finding and appears in disagreement with current belief on clinical outcome in patients carrying a single kidney.

**MO034**  
**INDUCIBLE RAT NEPHRIN TRANSGENE EXPRESSION IN PODOCYTES RESCUES NEPHRIN DEFICIENT MICE FROM PERINATAL DEATH**

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**Introduction and Aims:** Mutations leading to nephron loss abrogate podocyte function resulting in massive proteinuria both in humans and mice. Children born with nephrin deficiency die within the first year without kidney transplantation. Nephrin deficient mice die either in uterus or shortly after birth. This early perinatal lethality masks the role of the nephrin in adult podocytes and in extra renal tissues.

In order to test whether podocyte specific transgene expression of nephrin can rescue nephrin KO mice from perinatal lethality, we generated transgenic mice, selectively expressing a doxycycline inducible (tet-on) rat nephrin transgene in podocytes, and crossed these phenotypically normal mice with a nephrin deficient mouse line.

**Methods:** Doxycycline was administered to the F1 females via the drinking water (2.0 mg/ml in 5% sucrose) from conception on, and during pregnancy. After birth, doxycycline was administered via the drinking water (2.0 mg/ml in 5% sucrose) for one or six weeks. Thereafter the mice were sacrificed; the tissues were collected, weighed and prepared. The dissected tissues from one- and six-weeks-old mice of each genotype were analyzed by light-, fluorescence- and electron microscopy using standard procedures. mRNA and protein expressions were measured using RT-PCR and immunoblotting assays.

**Results:** In the presence of doxycycline, offspring that lacked endogenous nephrin but expressed rat nephrin from their transgene, survived to at least six weeks after birth while the nephrin deficient pups all died in uterus. The rescued mice were smaller, developed distinct histological abnormalities in the kidney, pancreas, testis and brain. In the kidney, these mice had changes in gene expression pattern and protein localizations. These proteins are known to be important in transcriptional regulation of nephrin and its signaling functions.

**Conclusions:** We have created a novel, transgenic mouse line expressing inducible rat nephrin cDNA in the podocytes. This nephrin transgene appeared to restore glomerular function at least partly in nephrin-deficient mouse embryos.

**MO035**  
**GENETIC PREDISPOSITION TO ATYPICAL HAEMOLYTIC URAEMIC SYNDROME**

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**Introduction and Aims:** Genetic and functional analyses have shown that complement regulation may be impaired in atypical haemolytic uraemic syndrome (aHUS) patients. Mutations in CFH (factor H), MCP (Membrane Coactor Protein) or CFI (factor I) genes have been found in about 50% of aHUS patients. Incomplete penetration of the disease in individuals carrying these mutations is relatively frequent.

We hereby report genetic analyses in four patients with aHUS and no familial history of the disease. All of them presented with acute renal failure with dialysis requirements, and neurological symptoms. Evidence of thrombotic microangiopathy was obtained in renal biopsies in all four patients. They all presented very low serum complement C3 levels at disease onset, and three of them also presented low C4 level. The four patients were treated with plasma exchange. One patient presented full recovery of renal function; the other three patients recovered enough renal function to be off dialysis but developed chronic renal disease and hypertension. After 41 (±29) months of follow-up, immunological and genetic studies regarding complement profiles were conducted on the four patients and their relatives.

**Methods:** Complement studies included quantification of C3, C4, factor H and factor I in plasma, expression of MCP on peripheral blood leukocytes, and sequencing of CFH, CFI and MCP genes. Plasma sample from the patients were also tested for the presence of factor H autoantibodies.

**Results:** No complement abnormalities were found in the aHUS patient who presented complete remission. Persistent low levels of C3 were observed in the other three patients. Decreased levels of factor H were detected in two patients. One of these patients carried two mutations in the CFH gene. The second patient carried a mutation in CFH and another mutation in the MCP gene.

**Conclusions:** Our results further illustrate that genetic abnormalities of complement regulatory molecules confer significant predisposition to aHUS, which in turn provides important insights into the aetiology of aHUS in these patients.
Acid base and ions transport

**THE ECTOPIC DEPOSITION OF α5/6 (IV) COLLAGEN CHAINS ARE NOT ASSOCIATED WITH THE RENAL IMPROVEMENT OBSERVED IN THE 129SVJ STRAIN OF MICE**

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Introduction and Aims: Alport syndrome is a well known hereditary disease which eventually results in irreversible nephritis. A recent paper identified the ectopic deposition of α5/6 (IV) collagen chains found in the glomerular basement membrane (GBM) of autosomal recessive Alport mice (Col4a3 knockout (KO) mice). It remains controversial whether this finding is related to a prolongation of the life span.

Methods: We therefore produced Col4a3 & 6 double knockout (DKO) mice between Col4a3 KO mice and Col4a6 & 6 DKO mice in order to investigate their renal function and life span in the 129svj strain of mice.

Results: We found no significant difference in the life span between Col4a3 KO mice and Col4a3 & 6 DKO mice. Both α5 and α6 (IV) collagen chains were found to be expressed in the GBM of Col4a3 KO mice, however, α3 and α4 (IV) collagen chains were not expressed in the GBM of Col4a3 KO mice. In addition, α3, α4, α5, and α6 (IV) collagen chains were not expressed in the GBM of Col4a3 & 6 DKO mice. These results suggested that the expression of the α5 (IV) collagen chain in the GBM required the presence of the α6 (IV) collagen chain in Alport mice.

Conclusions: In the 129svj mouse strain, the ectopic deposition of α5/6 (IV) collagen chains in the GBM did not demonstrate a sufficiently large volume to improve the life span.

**FIBRINOGEN AMYLOIDOSIS: REPORT OF THE PEACEFUL CLUSTER**

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Introduction and Aims: Hereditary nephropathic amyloidosis has been described as an autosomal dominant condition associated with mutations in apolipoproteins AI and AII fibrinogen Alpha chain (AFib) and lysozyme. Liver-kidney transplantation has been performed as a treatment for these diseases. AFib was first described in 1993; the point mutation at position 526 (AFib Glu526Val) was reported in less than 25 Northern European kindreds. The disease has a variable penetrance and can be present from childhood to old age. The aim of this study was to identify hereditary nephropathic forms of amyloidosis in Portugal.

Methods: Since 1985 we systematically evaluated Portuguese patients with amyloidosis, proteinuria and/or renal failure. Biopsy specimens were tested by immunohistochemistry. In the absence of AL, AA or TTR amyloidosis, the coding regions of APOA1, APOA2, FGA and LYZ were PCR amplified and sequenced.

Patients were examined and the family history was thoroughly detailed.

Results: In 12 patients (7M/5F) the Afib Glu526Val mutation was present. 11 heterozygous and 1 homozygous. We have not found other mutations. All probands were from five geographical areas, a region with 237,807 inhabitants in the district of Braga, north of Portugal. Their kindreds were not related to our knowledge. Genealogical evaluation revealed nephropathy in the relatives of 8 patients and 8 asymptomatic gene carriers. Pedigree analysis showed the presence of mutation or end-stage renal failure (ESRF) in 3 consecutive generations. Obligatory gene carriers were asymptomatic over the 80 years. The disease appeared between the 4th and 8th decade of life with a long history of hypertension, proteinuria and ESRF. The homozygous patient received a renal transplant and 11 years after no clinical recurrence of amyloid was evident. Extra-renal features occurred in 7 heterozygous and in the homozygous patient. Echocardiography showed left ventricular hypertrophy and left atrial enlargement in 5 patients and 1 required pacemaker implantation. Organomegaly, history of peptic ulcer and peripheral sensitive complaints were also documented. In 2 families history of ischemic stroke was present. The fibrinogen plasma concentrations were in the normal range and lower in the homozygous patient. Renal biopsy specimens were available in 7 patients. Amyloid deposits were mainly present in the glomeruli, but unexpectedly arteriomes and cortical interstitium were also involved. There was marked variation in the intensity of the staining with antifibrinogen antibiotics.

Conclusions: Portuguese AFib Glu526Val families show a typical geographical distribution suggesting that they may derive from a single founder. This represents the largest worldwide focus of disease. Clinical signs of extra-renal disease are frequent in our patients. Although simultaneous liver-kidney transplantation has been recommended the disease can be managed with an isolated kidney graft.

Acid base and ions transport

**PROGNOSTIC SIGNIFICANCE OF HYPERNATREMIA IN PATIENTS WITH SEVERE TRAUMATIC BRAIN INJURY**

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Introduction and Aims: Hypernatremia (HyperNa) is frequently observed in critically ill patients, and is associated with an increased risk of death. It is not known, however, whether this holds true also for patients with severe traumatic brain injury (TBI).

Methods: We analyzed prospective data from all patients admitted for severe TBI (GCS ≤ 8) to a tertiary care referral trauma ICU over a 3-year observation time period. We collected demographic, clinical variables and complications, as well as the available laboratory data for each day of ICU stay. Major outcomes were ICU and hospital mortality, and ICU length-of-stay (LOS). We used Cox proportional-hazards regression models with time-dependent variates designed to reflect the exposure to the varying Na levels over time during ICU stay. The same models were adjusted for age, gender, and sodium levels at admission as baseline covariates.

Results: We included in the study 130 TBI patients (mean age 52 yrs, SD 23, range 18-96; males 74%; median GCS 3, range 3-8; mean SAPS II 50, SD 14, range 9-84; all mechanically ventilated; tracheostomy in 64/130, 49%). ICU mortality was 36/130 (27.7%), hospital mortality 42/130 (32.3%). Follow-up included a total of 1334 patient-days, with an average of 2.9 measurements of serum Na per day. Serum Na values were computed as the daily average, which was 140 mmol/L (range 133 to 153); the patient average of the daily maximum Na levels was 143 mmol/L (range 131-164). Twenty-six percent of the days in ICU were complicated by HyperNa (i.e. at least one value of Na above 145 mmol/L), with 70% of the patients showing this abnormality. The average time of first occurrence of HyperNa was 5 day from ICU admission, while only 5 patients had HyperNa at ICU admission. A daily increase from the cumulative patient-average by 1 unit standard deviation (about 2.4 mmol/L of Na) was associated with an increased hazard of death of 2.15 times (95%confidence interval:1.28 to 3.59;P = 0.004). Adjustment for the daily use of hypertonic solutions did not change our findings. HyperNa was slightly associated with increased ICU LOS; in patients whose stay in ICU lasted less then 1 week, from 1 to <2 week, from 2 to <3, and ≥3 weeks, the proportion of days complicated by HyperNa was 15.3, 21.2, 27.7, 24.3% respectively (P = 0.028 for trend).

Conclusions: Our study suggests a strong relation between increased Na levels and mortality in patients with severe TBI. Though these results does not prove a causal relation between increased Na levels and death, we urge that interventional studies be designed to ascertain the safety of treatment strategies which might increase serum Na levels in patients with severe TBI.
Acid base and ions transport

MO039 CATION TRANSPORT ACTIVITY IN FUNCTIONAL ANION EXCHANGERS; A NOVEL PROPERTY OF DISTAL RENAL TUBULAR ACIDOYSIS (dRTA) ASSOCIATED ANION EXCHANGER 1 (AE1, BAND 3) MUTANTS

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Introduction and Aims: The anion exchanger 1 (AE1) is a membrane glycoprotein, which mediates the electroneutral exchange of chloride for bicarbonate across the cell membrane, and which is present in the erythrocyte and the α-intercalated cell in the distal nephron. Distinct mutations may cause red cell disease (hereditary spherocytosis and hereditary stomatocytosis, HSt) or renal disease (dRTA). Recent work has demonstrated that certain AE1 mutations associated with erythrocyte diseases, notably HSt, have a ‘cation leaky’ property that abolishes normal anion exchange and establishes an unselective cation transport property, causing the molecule to become leaky to potassium and sodium. We wanted to determine whether such a property was present in the dRTA associated AE1 mutants, which might theoretically mediate a pathological cation leak from the α-intercalated cell.

Methods: We examined both the anion and cation transport properties of a number of dRTA associated mutations, both European autosomal dominant (AD) AE1 mutants (R589H, G609R, S613F), and S.E.Asiatic autosomal recessive (AR) mutants (G701D, S773P, ΔV850, A858D); all co-expressed with glycophorin A (GPA - to enhance plasma membrane expression) in Xenopus laevis oocytes. Anion transport was assessed by chloride influx and intracellular pH measurements, cation transport by rubidium influx and intracellular cation content measurements.

Results: Anion exchange was preserved and similar to wild type (wt) in all the studied mutants. However, we demonstrated a cation flux in these mutants, significantly higher than wt in all the mutants, but much higher in the Asian AR mutants, most notably G701D. When we coexpressed the AR mutants with wt AE1 in the absence of GPA, we observed a ‘cation leak’ property that abolishes normal anion exchange and establishes an unselective cation transport property, causing the molecule to become leaky to potassium and sodium. We wanted to determine whether such a property was present in the dRTA associated AE1 mutants, which might theoretically mediate a pathological cation leak from the α-intercalated cell.

Conclusions: This work underlines how closely related AE1 is to a cation transporter and that anion exchange is independent of cation transport activity. Also, it suggests that heterozygotes for the AR dRTA mutations may have functionally ‘cation leaky’ AE1 at the cell surface of their red cells (P<0.05); Carba, 10-5M was added to the bath fluid.

Results: The densitometric analysis of the immunoblotting experiment showed an increase of 38.8% in AQP2 protein expression (control 100±4.3 vs Carba 138.8±12.1, P<0.05). In microperfusion, Carba 10-5M was added to the bath in Vaspreserin (Vp) absence (n=6) increased P from control, 12.3±3.6 to Carba, 62.6±14.8 (P<0.05) and recovery to 17.4±5.5 (P<0.05). In order to study the mechanism by which Carba activates the Vp cascade, we also used the Vp receptor inhibitor SR 121463A (a high potent and selective nonpeptide Vp receptor antagonist) (Anti-Vp2:n=6); control, 30.3±8.1; Carba, 52.6±15.0 (P<0.05); Carba+Anti Vp 2, 31.4±9.2 (P<0.05) and recovery, 26.2±5.9; and it was used the H8 inhibitor M-9656 of PKs (H8; n=4); control, 15.0±1.9; Carba, 106.10±12.3 (P<0.05); Carba+H8, 60.3±16.4 (P<0.05); and recovery, 44.5±13.2.

MO041 EFFECT OF A NEGATIVE REGULATION SEQUENCE OF GENOME EXPRESSION WITHIN 3'-UTR OF HUMAN SDCT2 ON mRNA STABILITY

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Introduction and Aims: Sodium-dependent dicarboxylate transporter (SDCT/NaDC) is an organic anion transport protein family responsible for transport of tricarboxylic acid cycle (Kreb cycle) intermediates and may involve in the regulation of aging and lifespan. High-affinity SDCT (SDCT2/NaDC3) is primarily expressed in kidney tissue. In addition, it is also expressed in several important organs with very active energy metabolism, such as liver, brain and placenta. Our previous studies have found that the expression level of human SDCT2 gradually increases with aging process and high glucose can up-regulate expression level of SDCT2. However, the molecular mechanism underlying the regulation of SDCT2 expression is not clear up to day.

Methods: In order to explore whether the 3’-untranslated region (3’-UTR) of SDCT2 gene plays a role in the regulation of SDCT2 protein expression, first of all, the characteristics of 3’-UTR sequence of SDCT2 gene was analyzed using bioinformatics. The results found that within the 3’-UTR of SDCT2 mRNA, there is a negative regulation region which can accelerate the degradation of SDCT2 mRNA and may play a regulation role for gene expression at post-transcriptional level. These results suggest that within the AU-rich region of 3’-UTR of SDCT2 mRNA, there is a negative regulation region which can decrease the stability of SDCT2 mRNA, accelerate the degradation of SDCT2 mRNA and may play a regulation role for gene expression at post-transcriptional level.

Results: The results showed that the AUR of SDCT2 could significantly reduce the expression level of GDP in the pDNA-GFP-AUR transfected cells (P<0.01). After blockade of RNA transcription with actinomycin D, total RNA was extracted from stably transfected HEK293 cells with an interval of 2 hours, and the stability of GFP-AUR and GDP mRNAs was analyzed by Northern blot. The results indicated that the stability of GDP-AUR mRNA is less than that of GFP mRNA. These results suggest that within the AU-rich region of 3’-UTR of SDCT2 mRNA, there is a negative regulation region which can decrease the stability of SDCT2 mRNA, accelerate the degradation of SDCT2 mRNA and may play a regulation role for gene expression at post-transcriptional level.

Conclusions: These results suggest that within the AU-rich region of 3’-UTR of SDCT2 mRNA, there is a negative regulation region which can decrease the stability of SDCT2 mRNA, accelerate the degradation of SDCT2 mRNA and may play a regulation role for gene expression at post-transcriptional level.

MO040 AQP2 EXPRESSION AND WATER ABSORPTION INCREASED BY CARBAMAZEPINE IN NORMAL RATS IMCD

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Introduction and Aims: Carbamazepine is an anticonvulsant and psychotropie medication commonly used in the treatment of people with intellectual disability (ID). This drug was used to try to decrease the urinary volume in Diabetes Insipidus because has an antidiuretic effect, but the incidence of the hyponatremia in neurological patients is a common occurrence. Therefore, the mechanisms whereby carbamazepine causes hyponatremia are not completely understood. Evidences that carbamazepine may increase the secretion of antidiuretic hormone (ADH) or may increase the renal sensitivity to vasopressin in the distal convoluted tubules and collecting ducts have been suggested. Our objective was investigated if Carbamazepine has a direct effect in the inner medullary collecting duct (IMCD) from normal rats.

Methods: In vitro study: 1) Immunoblotting studies for AQP2 protein expression in IMCD tubules suspension from normal rats incubated with Carbamazepina 10-5M by 30 minutes. 2) Microperfusion studies – the water permeability (PI, nm/sec) was determined in normal rats IMCD isolated and perfused by the standard methods. Carbamazepine (Carba) 10-5M was added to the bath fluid.

Results: The densitometric analysis of the immunoblotting experiment showed an increase of 38.8% in AQP2 protein expression (control 100±4.3 vs Carba 138.8±12.1, P<0.05). In microperfusion, Carba 10-5M was added to the bath in Vaspreserin (Vp) absence (n=6) increased PI from control, 12.3±3.6 to Carba, 62.6±14.8 (P<0.05) and recovery to 17.4±5.5 (P<0.05). In order to study the mechanism by which Carba activates the Vp cascade, we also used the Vp receptor inhibitor SR 121463A (a high potent and selective nonpeptide Vp receptor antagonist) (Anti-Vp2:n=6); control, 30.3±8.1; Carba, 52.6±15.0 (P<0.05); Carba+Anti Vp 2, 31.4±9.2 (P<0.05) and recovery, 26.2±5.9; and it was used the H8 inhibitor M-9656 of PKs (H8; n=4); control, 15.0±1.9; Carba, 106.10±12.3 (P<0.05); Carba+H8, 60.3±16.4 (P<0.05); and recovery, 44.5±13.2.

Conclusions: Our data showed that Carba increased the water permeability, probably acting directly in the Vp V2 receptor-Protein G complex, since its action was blocked by the specific Vp V2 receptor antagonist. These results could explain, at least in part, the hyponatremia observed in patients using this antiepileptic drug.
MO042 REGULATION OF THE RENAL Cl-/HCO3- EXCHANGERS AE1 AND PENDRIN
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Introduction and Aims: In the collecting duct, the Cl-/HCO3- exchanger AE1 (Band3, SLCA1A) is expressed on the basolateral membrane of type A intercalated cells and is critical for normal acid excretion and acid-base status. The apical anion exchanger pendrin (SLC26A4) is localized to type B cells and is thought to mediate bicarbonate secretion during alkalosis. Furthermore, pendrin may be important for chloride reabsorption and blood pressure regulation. The aim of this study was to investigate the regulation of AE1 and pendrin in response to changes in acid-base status, dietary electrolyte intake, and hormonal effects of mineralocorticoids.

Methods: C57BL/6 male mice were divided into 6 groups: group 1 control; group 2 0.28M NH4Cl; group 3 0.28M NaHCO3, and s.c. injections of 2mg of the aldosterone analogues DOCA (dehydroxy corticosterone acetate) mouse at day 1 and 4; group 4 0.28M NaCl; group 5 s.c. injections of 2 mg DOCA/mouse at day 1 and 4, and group 6 received 0.28M NaHCO3. All animals were given 1% sucrose in drinking water, treated for 7 days and placed in metabolic cages for the last 3 days. Western blotting and qRT-PCR were performed separately on cortex and medulla.

Results: Urine pH was markedly increased in all 3 groups treated with DOCA/NaHCO3, DOCA or NaHCO3, respectively. In contrast, urine pH was significantly lower in the NH4Cl treated mice. AE1 mRNA and protein expression in kidney cortex was not changed in all treated groups. In contrast, treatment of mice with NH4Cl, NaCl, NaHCO3 or NaHCO3/DOCA increased AE1 protein expression. mRNA abundance was also increased in response to NaCl, NaHCO3 or NaHCO3/DOCA but not during NH4Cl loading suggesting different regulatory mechanisms. Pendrin protein expression was reduced by NaCl or NH4Cl loading but remained unaffected by all other treatments. Similarly, NH4Cl reduced pendrin mRNA abundance, whereas NaCl loading increased pendrin mRNA, which may be part of a compensatory reaction.

Conclusions: Both AE1 and pendrin are strongly affected by acid-base status and sodium chloride intake. However, pendrin appears to be more sensitive to changes in chloride whereas AE1 seems to respond mainly to sodium. Acidosis regulates both transporters. Interestingly, protein and mRNA levels are not regulated in parallel in all instances suggesting specific regulatory mechanisms. The link between both transporters and renal salt handling and possibly blood pressure regulation requires further investigations.

Dialysis 2

MO043 ANTIMICROBIAL LOCK, ANTIBIOTIC OINTMENT AND CATHETER RELATED-INFECTION
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Introduction and Aims: The percentage of patients using a central venous catheter as their first haemodialysis access and thereafter as a prevalent vascular access has grown steadily. The early exhaustion of all vascular territories, in an ever-aging patient population with high rates of diabetes, hypertension and other vascular comorbidities, accounts for this apparent overuse of cuffed tunnelled dialysis catheters as long-term dialysis access, which affects more than 28% of all patients in many units. The rate of infectious morbidity and mortality is much higher when catheters are used than when patients are dialysed through grafts or native fistulas.

Methods: The authors undertook a prospective, randomised, open-label, clinical trial during which they compared the efficacy of dressing the exit site with antibiotic ointment (AO) vs catheter antimicrobial locking (AL) in preventing catheter-related infection (CRI). Standard precautions and appropriate catheter care were adopted in every patient.

Results: A total of 147 tunnelled catheters, newly implanted in 122 consecutive patients, were followed during a 2-year period. Patients were randomly distributed into one of three groups: (a) Group A received AO prophylaxis; (b) Group B was treated with a heparin + gentamicin (5.2 mg/ml) lock (AL); and (c) Group C received both AO and AL prophylaxis. Group A had a significantly lower infection-free time (p<0.002), with a reduced catheter survival of 99.7 days and a significantly higher number of catheter-related infections (15 episodes, p<0.002). Group B had 129.1 mean infection-free days and 3 episodes of catheter-related infection. Group C had 127.3 mean infection-free days and 5 episodes of catheter-related infection. There was no difference in the CRI rate between groups B and C. No toxicity or other adverse events were observed during this two-year period and the efficacy of the preventive measures remained stable throughout.

Conclusions: The authors concluded that antimicrobial lock is superior to antibiotic ointment as a CRI preventive measure and there is no additive effect when both methods are associated.

MO044 USING THE AMOUNT OF AMNIOTIC FLUID AS AN INDEX TO HAEMODIALYSIS ADEQUACY IN CHRONIC RENAL FAILURE PREGNANT WOMEN
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Introduction and Aims: The incidence of polyhydramnios (PH) in chronic renal failure (CRF) pregnant women undergoing hemodialysis (HD) is as high as 50%, being the same incidence in general preganat population around 1%. PH is associated with a 3 times greater risk of premature delivery, which is the most important pregnancy complication in CRF women. Increased fetal urine production, secondary to urea osmotic diuresis, is probably the etiology of the excess of amniotic fluid volume. Therefore, reducing maternal blood urea level by increasing hemodialysis dose, may act reducing fetal urine flow rate, yielding the correction of the amount of amniotic fluid. The aim of this study is to demonstrate that PH can be treated with increasing HD dose and to propose PH as a tool to adequate HD in chronic renal failure pregnant women.

Methods: In the last 7 years we have prospectively followed up 32 pregnant women that required HD for at least 15 days, with a 90% fetal survival rate. Nine patients developed an excess of amniotic fluid, defined as an amniotic fluid index (AFI) > 18 cm, or in gemellar pregnancies, as the deepest vertical pocket (DVP) > 6 cm. All pregnant women were submitted to a tailored short high efficiency daily HD protocol. Patients with less than 1 year on dialysis and more than 1000 mL of diuresis, started a 90 minutes, 6 times a week HD protocol. Patients with more than one year on HD or less than 1000 mL of diuresis were putted on an initial 120 minutes, 6 times a week regimen. Pregnant women with AFI > 18 cm or DVP > 6 cm had the HD dose elevated by increasing the dialysis time in half an hour.

Results: All patients with excess of amniotic fluid treated with the increasing of HD dose have normalized the amniotic fluid volume within 15 days. PH relapsed in 2 patients 20 and 30 days after the first change in HD dose. These patients were treated again, with an half an hour increasing in HD time, with normalization of the AFI. The mean AFI ± SD before and after the change in HD dose were, 24.8±6.0 and 17.5±2.4 cm, respectively (p<0.005, paired Student t test). The mean predialysis blood urea level before and after the change in HD dose were, 96.2±33.0 and 71.1±27.7 mg/dl, respectively (p<0.05 paired Student t test). The 9 pregnancies with elevated AFI or DVP resulted in 11 live infants (2 gemellar pregnancies), with mean gestational age and weight of 34±2.2 weeks and 1996±488 g, respectively. The gestational age and fetal weight of the patients with excess of amniotic fluid do not differ from the remaining 23 patients (gestational age 34±3.0 weeks, and weight 1841±617g, respectively).

Conclusions: The excess of amniotic fluid volume in CRF pregnant women, undergoing a daily high efficiency hemodialysis regimen, can be treated increasing HD dose. The presence of PH, AFI > 24 cm or excess of amniotic fluid, AFI > 18 cm, are an indication of dialysis inadequacy and the dialysis dose should be enhanced.
EFFECT OF GLUCOSE DEGRADATION PRODUCTS, COMPARISON OF PROGRESSION RATES OF CORONARY ARTERY CALCIFICATIONS ON AQPI AND ENOS EXPRESSION IN CULTURED ENDOTHELIAL CELLS

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Introduction and Aims: Aquaporin-1 (AQP1) and endothelial NO synthase (eNOS) expression on the endothelium of peritoneal vessels modulate ultrafiltration during peritoneal dialysis (PD) by different mechanisms. Protracted AQPI and eNOS activation may, in the long term, be deleterious for the peritoneal function. We aimed at examining the effect of peritoneal dialysis solutions (PDS) and glucose degradation products (GDPs) on the expression of AQPI and eNOS in cultured endothelial cells.

Methods: An endothelial cell line (t End.1) was incubated for 24h with two GDPs (2-furaldehyde –Fur- or methylglyoxal - MGly- at concentrations found in traditional PDS significantly upregulated eNOS mRNA (Fur: 159.9 ±21.8, both p < 0.05 versus untreated control cells) and tended to downregulate AQPI mRNA (Fur: 98.8±20.6%; MGly: 87.4±22.6%; p non-significant) in cultured endothelial cells. Glucose-based PDS as well as icodextrin PDS significantly upregulated basal AQPI mRNA (1.36% glucose PDS: 136.5 ±20.6%; Icodextrin: 156.6 ±15.4%; p<0.05 versus untreated control cells) and eNOS mRNA (1.36% glucose PDS:136.5±20.6%; MGly: 87.4±22.6%; p non-significant) in cultured endothelial cells.

Results: Fur and MGly at concentrations reported in traditional PDS significantly upregulated eNOS mRNA (Fur: 159.9 ±21.8, both p < 0.05 versus untreated control cells) and tended to downregulate AQPI mRNA (Fur: 98.8±20.6%; MGly: 87.4±22.6%; p non-significant) in cultured endothelial cells. Glucose-based PDS as well as icodextrin PDS significantly upregulated basal AQPI mRNA (1.36% glucose PDS: 136.5±20.6%; Icodextrin: 156.6 ±15.4%; p<0.05 versus untreated control cells) and eNOS mRNA (1.36% glucose PDS:136.5±20.6%; MGly: 87.4±22.6%; p non-significant) in cultured endothelial cells.

Conclusions: In cultured endothelial cells, all PDS triggered both AQPI and eNOS in a likely feed-back mechanism. GDPs stimulated e-NOS expression only, and this effect might favor in the long-term PD ultrafiltration failure.

Baseline CACs was lower in patients treated by 8-h HD for at least six months compared to conventional HD patients but difference did not reach significance (median CACs 312 and 468). CACs significantly increased during follow-up in both groups, progression rates were not different (CACs 116 and 127 in 8-h and 4-h group). In patients with baseline CACs higher than 200, progression rate was significantly lower in 8-h HD group than 4-h HD group (median CACs 141 (67-291) and 372 (142-695), p<0.001). CACs was positively correlated with pre- and post-dialysis uric acid (r: 0.42 and r: 0.43) and creatinine (r: 0.33 and r: 0.39), phosphate (r: 0.42), CaX product (r: 0.41), phosphate-binder dosage (r: 0.46) and inversely correlated with serum bicarbonate (r: -0.34), Kt/V (r: -0.39), and duration of HD session (r: -0.35). In multivariate analysis, serum phosphate level was independent predictor for the progression of CACs.

Introduction and Aims: Aquaporin-1 (AQP1) and endothelial NO synthase (eNOS) expression on the endothelium of peritoneal vessels modulate ultrafiltration during peritoneal dialysis (PD) by different mechanisms. Protracted AQPI and eNOS activation may, in the long term, be deleterious for the peritoneal function. We aimed at examining the effect of peritoneal dialysis solutions (PDS) and glucose degradation products (GDPs) on the expression of AQPI and eNOS in cultured endothelial cells.

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Conclusions: In cultured endothelial cells, all PDS triggered both AQPI and eNOS in a likely feed-back mechanism. GDPs stimulated e-NOS expression only, and this effect might favor in the long-term PD ultrafiltration failure.

Impact of Dietary Salt Intake on Peritoneal Membrane Transport and Structure in Rats

Introduction and Aims: Dietary salt intake has been linked to enhanced progression of renal fibrosis by upregulation of TGF-β. In PD patients, intraperitoneal glucose administration leads to upregulation of TGF-β and enhanced mesothelial-fibroblast transition. As patients with a high salt intake also need more hyperosmotic glucose, it is important to know whether high salt intake also induces changes in the peritoneal membrane.

Methods: 12 healthy non-uremic Wistar rats were randomised to NS (normal salt) or HS (high salt). All rats received normal rat chow. NS rats had free access to tap water, HS rats had only free access to NaCl 2% as drinking water. After two weeks, rats were subjected to an abbreviated rat PET test. Animals were injected IP with 15ml of 3.86% glucose solution at time zero. After 60 (N=26) and 120 minutes (N=26), dialysate was sampled for determination of sodium, glucose, and urea. IP volume was determined by complete collection of IP fluid by depping with pre-weighed gauses. Visceral and parietal peritoneal tissue were sampled for determination of TGF-β mRNA.

Results: In the NS vs the HS, IP urea was 0.54±0.06 vs 0.60±0.05 (p=0.08) at 60 and 0.68±0.05 vs 0.77±0.04 (p=0.009) at 120mins. In the NS vs the HS, D/D0 glucose was 0.23±0.03 vs 0.21±0.04 (p=0.3) at 60 and 0.12±0.02 vs 0.11±0.03 (p=0.6) at 120mins. In the NS vs the HS, IP volume was 24.6±0.6 vs 24.3±0.7ml (p=0.5) after 120 minutes. When visceral peritoneum of the NS rats was taken as standard, HS rats had a 2.4±1.4 fold (visceral), and a 1.58±0.36 fold (parietal) increase for TGF-β mRNA production.

Increased dietary salt intake during two weeks induced in normal non-uremic rats a significant increase in small solute transport, and a non significant increase in glucose absorption. There was no difference between the groups in IP volume after 120 minutes. Although a bit disappointing, it should be taken into account that exposure time was only two weeks, and that we used non-uremic animals. There was a clear upregulation of TGF-β mRNA production in the salt loaded rats, and this both at the visceral and peritoneal peritoneum.

The experiments need to be repeated in uremic rats, and with longer...
exposure times, as the impact of salt loading might be more enhanced in uremic animals, as e.g. for renal scarring.

From the current results, it is difficult to differentiate whether salt exposure induced a real discrepancy between small solute transport and glucose transport, or whether the study lacked power to find a significant difference in glucose transport. Further anatomicopathologic analysis of the samples is warranted. Neither is it clear whether the lack of difference in IP volume was related to the very large IP volumes, making hydraulic forces overcome osmotic ones.

**Conclusions:** An enhanced dietary salt intake in non uremic rats during 2 weeks, induced an increase in small solute transport, but not a decrease of ultrafiltration. There was an upregulation of TGF-β mRNA expression in the salt loaded animals.

**Disclosure:** Ameleen P. Letincick has received an unrestricted research grant from Fresenius Medical Care Europe for animal model research.

**Cardiovascular diseases 2**

**MO048 ELEVATED ENDOGENOUS ERYTHROPOIETIN-LEVELS ARE ASSOCIATED WITH INFLAMMATION IN PATIENTS WITH DIABETES MELLITUS AND CHRONIC KIDNEY DISEASE**

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**Introduction and Aims:** Anemia is prevalent in patients with diabetes mellitus and is associated with increased cardiovascular morbidity and mortality. Anemia is also common in advanced stages of chronic kidney disease (CKD). In patients with diabetes and CKD, the pathogenesis of anaemia and the role of endogenous erythropoietin (EPO) remain uncertain, with relative EPO-deficiency as a possible mechanism. We evaluated the role of inflammation as a mediator of EPO-levels in this population.

**Methods:** For this cross-sectional analysis, we enrolled 252 patients with type 2 diabetes mellitus between 2004 and 2005. Patients were from four nephrology outpatient clinics in the Wuerzburg area, median age was 67 yrs, 51% were male, and median duration of diabetes was 10 yrs. Patients receiving dialysis, post kidney transplantation, or on iron/vitamin B12/folate-therapy, or on EPO-stimulating agents were excluded. Anemia was defined as hemoglobin <12 g/dl in women and <13.5 g/dl in men. GFR was calculated from the average of a 24h-creatinine- and urea-clearance. EPO-levels were measured by ELISA. Log-transformed CRP, ferritin and albumin were used as markers of inflammation. Associations between continuous variables were calculated with Pearson’s and Spearman’s correlation, and predictors of EPO-levels were evaluated using multivariable linear regression.

**Results:** The distribution of kidney function was as follows: CKD-stage 1: 6%, stage 2: 30%, stage 3: 36%, stage 4: 20%, stage 5: 8%, with a median GFR of 50 ml/min/1.73m². The prevalence of anemia was 43% in the total cohort and increased progressively from CKD stage 1 to 5 (14%, 15%, 44%, 78% and 94%, respectively). GFR was positively correlated with hemoglobin (r=0.52, p<0.0001) and negatively correlated with log-CRP (r=-0.21, p<0.0001) and log-albumin (p<0.001). After adjusting for age, sex, hemoglobin and proteinuria, logCRP (p<0.0001) and albumin (p=0.002) and ferritin (p=0.03) were independent predictors of EPO-levels. The effect of GFR as a predictor of EPO-levels became nonsignificant (p=0.1) when accounting for the described inflammatory markers.

**Conclusions:** In diabetic patients with CKD, anemia is prevalent and increases with declining GFR. Compared to early stages of CKD, EPO-levels were elevated in advanced CKD stages and independently associated with markers of inflammation (CRP, albumin and ferritin). A possible explanation for anemia with concomitantly elevated EPO-levels may be EPO-resistance due to inflammation rather than EPO-deficiency in this setting of diabetic patients with CKD.

**Disclosure:** Roche, Germany funded measurement of erythropoietin levels. Otherwise Roche, Germany did not provide any input in the conduct of the analysis as well as on reporting of the results.

**MO049 ASSOCIATION OF INSULIN RESISTANCE WITH DE NOVO CORONARY STENOSIS AFTER PERCUTANEOUS CORONARY ARTERY INTERVENTION IN HEMODIALYSIS PATIENTS**

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**Introduction and Aims:** De novo coronary atherosclerosis as well as restenosis may be involved in the poor prognosis after percutaneous coronary artery intervention (PCI) in hemodialysis patients. We investigated the incidence of de novo coronary stenosis after PCI and aimed to clarify the factors associated with de novo coronary stenosis.

**Methods:** We enrolled 106 patients on chronic hemodialysis (72 men, 34 women; mean age, 65.4±8.9 y), who had firstly received PCI with bare-metal stents for single coronary lesions and undergone follow-up coronary angiography (CAG) 6 months thereafter. Coronary lesion with stenosis of >50% diameter that was newly recognized at follow-up CAG was defined as de novo coronary stenosis. The values of biochemical parameters were determined as the means of several measurements during the 6 months between PCI and follow-up CAG.

**Results:** Follow-up CAG revealed de novo coronary stenosis in 40 (37.7%) of the 106-hemodialysis patients who had received PCI. Stepwise multiple logistic regression analysis showed that de novo coronary lesions were strongly associated with homeostasis model assessment insulin resistance index (HOMA-IR, 1 mM-[µU/ml]: odds ratio, 7.312; P = 0.001). This association of HOMA-IR with de novo coronary lesions was not influenced by Framingham coronary risk factors or factors peculiar to dialysis (Table).

**Conclusion:** Insulin resistance may be involved in the progression of nonculprit coronary atherosclerosis after PCI in hemodialysis patients.

**MO050 ASSOCIATION OF URIC ACID WITH CARDIOVASCULAR EVENTS AND MORTALITY IN HEMODIALYSIS (HD) PATIENTS: INTERNATIONAL RESULTS FROM THE DOPPS**

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**Introduction and Aims:** Hyperuricemia has been shown to be associated with hypertension, coronary artery disease and chronic kidney disease (CKD). However, there are limited data on the relationship of uric acid with mortality and cardiovascular events in HD patients.

**Methods:** Data from 16,535 randomly selected patients on chronic HD from 12 countries in the Dialysis Outcomes and Practice Pattern Study (DOPPS) were analyzed based upon the initial cross-section of patients in DOPPS I and II (1996-2004). Multivariable logistic regression examined predictors of high vs. low uric acid levels, and Cox regression was used to determine the relative risk (RR) of all-cause mortality and CV-related mortality and events. All models were adjusted for age, gender, race, body mass index.
CAROTID ARTERY INTIMA-MEDIA THICKNESS AND MORTALITY IN HEMODIALYSIS PATIENTS: THE KEY ROLE OF INFLAMMATION

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Introduction and Aims: The impact of carotid artery intima-media thickness (CA-IMT) in conjunction with C-reactive protein (CRP) on patient survival was investigated in a large group of hemodialysis (HD) patients.

Methods: Between September-November 2005, B-mode ultrasonography was performed to measure CA-IMT by the same operator in 531 prevalent HD patients with arteriovenous fistula as vascular access. All subjects (mean age 57±13 year, HD duration 57±45 months, 21% diabetes, 43% female) were prospectively followed for 18 months; all-cause mortality was evaluated. Demographical, clinical and time-averaged laboratory data were assessed.

Predictors of mortality were estimated by Cox regression, cumulative survival was analyzed by Kaplan-Meier method.

Results: During a mean 18±5 months of follow up, 49 deaths occurred (9.2%). Mean CA-IMT was higher in patients who died than patients alive (0.92±0.27 vs 0.78±0.20 mm, p<0.001).

IMT was positively correlated with age (r=0.54, P<0.0001), pulse pressure (r=0.11, p<0.05), hsCRP (r=0.21, p<0.001) and negatively correlated with albumin (r=-0.19, p<0.001).

Mortality rate was significantly higher in patients with CA-IMT>0.85 mm compared to patients with CA-IMT 0.70-0.85 and <0.70 mm (p<0.01). The risk for death was 11.6, 4.3 and 2.9/100 patient years in these tertiles, respectively.

In patients with hsCRP ≥0.5 mg/dl, survival rate was similar in CA-IMT tertiles. However, in patients with hsCRP >0.5 mg/dl, mortality rate was significantly increased by higher tertiles (3.7, 4.7 and 12.5/100 patients years, p<0.05).

In multivariate analysis, age, interdialytic weight gain (IDWG), body mass index (BMI), serum albumin, hsCRP and CA-IMT were independent predictors of mortality (table). An increase in CA-IMT per 0.1 mm was associated with 4.5-fold increase in mortality.

Conclusions: The association of higher uric acid with lower mortality is in contrast to its association with higher cardiovascular risk in the general population. While the analyses adjusted for numerous covariates, the possibility of residual confounding and potential selection bias merit further investigation.

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Mortality predictors in Cox regression analysis

| ExpB (95%CI) | p   |
|--------------|-----|
| Age          | 1.03 (1.00-1.07) | <0.05 |
| HD duration  | 1.38 (1.02-1.87) | <0.05 |
| CA-IMT       | 4.49 (1.9-16.9)  | <0.05 |
| BMI          | 0.89 (0.82-0.97) | <0.001 |
| Albumin      | 0.16 (0.05-0.50) | <0.05 |
| hsCRP        | 1.16 (1.05-1.28) | <0.01 |

CA-IMT and mortality in HD patients with hsCRP ≤0.5 mg/dl.

Conclusions: Association of increased CA-IMT used as a surrogate marker of “atherosclerosis” and increased CRP level reflecting “inflammation” leads to poor survival in HD patients.

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PROGRESSION OF CORONARY ARTERY CALCIIFICATION (CAC): COMPARISON BETWEEN DIALYSIS (HD) AND TRANSPLANT PATIENTS (Tx)

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Introduction and Aims: Progression of coronary calcification in HD, as evidenced by cardiac Multislice Computer Tomography (MSCT), has been related, at least partially, to divalent ions abnormalities and chronic
Hypertension awareness, treatment and control in individuals with chronic kidney disease: an analysis from the NKF-KEEP database

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Introduction and Aims: Hypertension is a major risk factor for chronic kidney disease (CKD). Several studies examined hypertension prevalence, awareness, treatment, and control rates in the general population but relevant data in CKD patients are limited. The objective of this study was to determine hypertension awareness, treatment, and control, and determinants of blood pressure control in individuals with CKD.

Methods: This is a cross-sectional analysis in subjects with CKD from the National Kidney Foundation-Kidney Early Evaluation Program (NKF-KEEP). KEEP is a national-based health screening program for individuals at high-risk for CKD conducted in 49 States and Columbia District. From a total of 55,220 participants as of December 2005, we examined 10,813 adults with CKD who had completed information for demographic and medical characteristics were used in the analysis. Proportions of prevalence, awareness, treatment, and control of hypertension were estimated. Predictors of blood pressure control were assessed using multiple logistic regression analysis.

Results: Hypertension prevalence, awareness and treatment proportions in the total population were high (86.2%, 80.2%, and 70.0% respectively) but control rate was low (13.2%). All these proportions were gradually increasing with advancing CKD stages. Systolic blood pressure accounted for most of inadequate control. Male gender (odds ratio [OR], 0.86; 95% confidence) MCT in [14], 0.75-20599), non-Hispanic black race (OR, 0.76; 95%CI, 0.65-0.89), and BMI≥30 kg/m² (OR, 0.83; 95%CI, 0.73-0.94) were negatively associated with control, whereas current smoking (OR, 1.30; 95%CI, 1.03-1.63), higher education (OR, 1.18; 95%CI, 1.00-1.39), having a doctor (OR, 1.86; 95%CI, 1.25-2.75) and increased CKD stages were associated with adequate control.

Conclusions: Despite increased awareness and treatment of hypertension, control rates in these participants is poor. This poor control rate centers around elevated systolic pressure in people who are obese, non-Hispanic black, or male. These data suggest that those who are aware of their kidney disease are more likely to achieve blood pressure control.

Vascular physiology and hypertension

THE ROLE OF MELATONIN IN THE ABNORMAL CIRCULATORY BLOOD PRESSURE RHYTHM IN PREECLAMPSIA

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Introduction and Aims: The non-dipping pattern of circadian blood pressure is a common finding in pregnant women with preeclampsia. These women have a greater cardiovascular morbidity and an increased frequency of adverse maternal and fetal outcomes. Melatonin, a powerful endogenous antioxidant, plays a pivotal role in the biologic regulation of circadian rhythms. In the present study we investigated the possible contribution of melatonin in the pathophysiology of the non-dipping blood pressure pattern in preeclampsia.

Methods: Twenty women with normal pregnancy (mean age 29.5±5.7 years) and 31 women with preeclampsia (mean age 29.1±5.5 years) underwent 24h ambulatory blood pressure monitoring and were categorized as non-dippers (when they exhibited a less than 10% decline of the nocturnal mean arterial pressure -MAP- relative to the daytime MAP) or dippers (>10% decline of the nocturnal MAP). Serum melatonin and urine 6-sulphatoxymelatonin (6-SMT; the main melatonin metabolite) were determined in daytime and nighttime blood samples and urine collections respectively, with commercially available ELISAs. In women with preeclampsia the measurements were repeated 2 months after delivery.

Results: Based on the 24h Blood pressure monitoring, 21 women with preeclampsia, but none of the controls, were categorized as non-dippers. Compared to healthy pregnant women, patients with preeclampsia showed significantly lower levels of nighttime serum melatonin concentrations (85.4±2.9 vs. 48.4±2.47 pg/mL, p<0.01) and nighttime urinary 6-SMT excretion (43.8±28.3 vs. 19.7±19.6 ng/mL, p<0.01). In addition, significantly lower levels of nighttime serum melatonin (37.5±19.8 vs. 71.2±17.5 pg/mL, p<0.01) and nighttime urinary 6-SMT (11.4±14.8 vs. 36.9±17.4 ng/mL, p<0.01) were found in non-dippers compared to dippers with preeclampsia. Reevaluation of the non-dipping group (n=21) 2 months after delivery showed a sustained non-dipping pattern in 11 and normalisation of the blood pressure rhythm in 10 women. The reappearance of the normal blood pressure rhythm coincided with the normalisation of the melatonin rhythm in all 10 women.

Conclusions: Nocturnal melatonin secretion is impaired in preeclamptic women with non-dipping pattern of circadian blood pressure compared to preeclamptic women with normal nocturnal blood pressure dip and normotensive pregnant women. In addition, women with a recent history of preeclampsia and nocturnal hypertension who continued to exhibit an impaired circadian rhythm of their blood pressure for at least two months after delivery retained also an impaired melatonin secretion. These findings provide evidence that melatonin may play a role in the altered circadian rhythm of blood pressure in preeclampsia and propose that melatonin could be associated with the mechanisms linking preeclampsia with increased cardiovascular risk.
DIETARY SALT INTAKE WAS RELATED TO MARKERS OF INFLAMMATION AND PROTEINURIA IN PRIMARY HYPERTENSIVE PATIENTS

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Introduction and Aims: Inflammation may play a critical role in the pathogenesis of hypertension and cardiovascular disorders such as atherosclerosis. Microalbuminuria has been associated with subclinical organ damage in non-diabetic hypertensive patients. Recently it was proven as a predictor of cardiovascular mortality in long-term studies. Even though the pathogenesis of increased urinary albumin excretion is at present still unclear. The aim of this study was to evaluate the relationship between salt intake and inflammation and albuminuria in nondiabetic patients with primary hypertension.

Methods: Two hundred ninety hypertensive patients (175 male and 115 female, mean age; 53±11 years) without diabetes were recruited in the study. Ambulatory blood pressure measurements, 24-hour urinary excretion of sodium (24 h UNa +) and albumin, serum creatinin, BUN, glucose, electrolytes, albumin, hemoglobin, lipid levels, inflammatory markers (homocysteine, fibrinogen, C reactive protein (CRP), erythrocyte sedimentation rate (ESR), lipoprotein-a) were evaluated. Patients were stratified into two groups according to 24 h U Na+: low sodium intake (<120 mEq/day; group I= 151 patients), high sodium intake (>120 mEq/day; group II=139 patients).

Results: No difference was observed demographic findings such as age, gender, dietary habits and number and class of anti-hypertensive agents. In group-II, the mean value of CRP was significantly higher than patients in group-I (0.60±0.58 vs. 0.44±0.32 respectively p<0.05). High sodium intake showed also a positive relationship with urinary albumin excretion. Amount of albumin excretion in group-II was higher than in group-I (60.3±11.6 respectively p<0.05). Systolic and diastolic blood pressure, glomerular filtration rate, hemoglobin levels, CRP, ESR, lipoprotein-a and lipid levels did not show significant variation and difference between groups.

Conclusions: The present study may support the hypothesis that high sodium intake is associated with inflammation and albuminuria in primary hypertensive patients. Our findings suggest that high sodium intake may cause systemic inflammation and albuminuria.

MO056 ★ ACHIEVEMENT OF RECOMMENDED BLOOD PRESSURE GOALS IN CHRONIC KIDNEY DISEASE IMPROVES ARTERIAL STIFFNESS

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Introduction and Aims: Patients with chronic kidney disease (CKD) have higher arterial stiffness (higher augmentation index (AIx)) which is associated with a higher risk for major adverse cardiovascular events. Achieving goal blood pressure (BP) is crucial in lowering the risk for MACE and CKD progression. European Society of Hypertension-European Society of Cardiology guidelines recommended that target BP for the CKD population should be at least <130/80 and at least <140/90 mmHg in all hypertensive patients. This study was conducted to examine whether achievement of goal BP improve arterial stiffness in the CKD population.

Methods: We reviewed the medical records of 283 patients who underwent standard BP measurement and applanation tonometry for estimation of AIx at the renal outpatient clinic from August 2006 to September 2007. 173 (61%) patients had HTN and CKD, and 110 had only HTN. The BP goal in the CKD group was <130/80 mmHg and for the non-CKD group was <140/90 mmHg. Continuous variables including age, eGFR, AIx, peripheral pulse pressure (PPP), BP, and number of antihypertensive medications were compared between groups using Wilcoxon rank-sum test.

Categorical variables including race, gender, DM and HTN were compared using Chi square and Fisher’s exact tests.

Results: The sample included 135 (48%) women, 195 (69%) white patients. Mean age was 57±16 years. 259 (92%) of patients were on antihypertensive medications; 71% patients were on 2 or more medications. There was no difference in the number of medications in the CKD compared to the non-CKD patients. 32% of CKD patients achieved BP goal and 48% of non-CKD patients achieved BP goal. Both CKD and non-CKD patients had similar demographic and clinical characteristics except lower systolic BP in the CKD patients (median systolic BP 128 mmHg [118, 146] vs 138 mmHg [126, 153], p=0.003) and less women in the CKD patients (42% vs. 57%, P=0.01).

The PPP and AIx in CKD patients with BP goal of <130/80 mmHg was significantly lower than that of patients who did not achieve this BP goal, Table 1. In patients with no CKD, there was no statistical difference in AIx for patients who achieved BP goal compared to patients not achieving BP goal, Table 1.

| Table 1 |
|-----------------|-----------------|-----------------|
| BP (mm Hg)      | CKD P-value     | Non-CKD P-value |
| <130/80         | ≥130/80         | <140/90         | ≥140/90         |
| [quarters]      | [percentiles]   | [percentiles]   | [percentiles]   |
| [36,51]         | [42,76]         | [42,56]         | [58,86]         |
| [12,27]         | [15,29]         | [16,32]         | [19,32]         |

Conclusions: This study reports that lowering BP to the recommended goal of <130/80 mmHg in patients with CKD improves AIx which reflects reduction in arterial stiffness. This may explain the mechanism by which there is a reduction in cardiovascular morbidity and mortality in these patients. In hypertensive patients without CKD, a more aggressive lower BP goal may be required to improve AIx.