Free Communications

**OSa001** ALLOPURINOL IMPROVES VASCULAR FUNCTION IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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Introduction and Aims: Oxidative stress (OS) causing endothelial dysfunction (ED) is thought to be a major reason why renal patients suffer more cardiovascular (CV) events than would be expected from their conventional risk factors such as BP. One way to reduce OS is to prevent its formation by using allopurinol to block xanthine oxidase (XO)-induced OS. In this study, we examined if allopurinol really does improve ED in renal patients.

Methods: A randomised, double-blind, placebo-controlled, parallel study was conducted in 67 patients with chronic kidney disease (CKD) stage 3. Subjects received 100mg Allopurinol once a day for the initial 2 weeks, and then increased to 300mg Allopurinol once a day for the remaining 9 months, or placebo. Endothelial function was assessed by flow-mediated dilatation (FMD) of the brachial artery, while central arterial stiffness was assessed by pulse wave analysis (PWA) and pulse wave velocity (PWV).

Results: 50 patients completed the study (24 active, 26 placebo). Mean age, estimated glomerular filtration rate (eGFR) and clinic BP were 72±8 years, 45±11 ml/min/1.73m², and 144/74 (± 18/8) mmHg respectively. Allopurinol significantly improved brachial artery FMD [Δ FMD was +1.3% (± 3.1%) in the active group and -0.8% (±2.9%) in the placebo group (p=0.020)]. There was no difference in response to glyceryl trinitrate between the two treatment arms. The central augmentation index (AIx) improved (p=0.020). PWV also significantly increased (p=0.0001, χ²-test). PWV was -0.3 m/sec (9.9%) in the active group and +0.4 m/sec (±1.5) in the placebo group (P=0.048). Renal function remained stable in both groups throughout the whole study period.

Conclusions: This is the first study to demonstrate that in patients with mild to moderate CKD, treatment with allopurinol significantly improved endothelial function when assessed using 3 contemporary but different modalities. As ED is an important surrogate marker that predicts future CV events, allopurinol could potentially reduce the adverse prognosis associated with renal dysfunction.

**OSa002** SILENT CEREBRAL ISCHEMIC LESIONS IN PREDIALYSIS PATIENTS WITH CHRONIC KIDNEY DISEASE

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Introduction and Aims: Patients with chronic kidney disease (CKD) have more risk factors for cerebrovascular diseases than general population. Magnetic resonance imaging (MRI) of brain is highly sensitive for detecting ischemic cerebral lesions. Ischemic cerebral lesions, such as silent cerebral lacunar infarction (SCI) and paraventricular hypertensity (PVH), have been reported to be risk factors for future cerebrovascular events and to be associated with dementia. In the present study, we investigated the prevalence of SCI and PVH in CKD patients without dialysis therapy, and examined the relationship between the prevalence of SCI, PVH and clinical factors.

Methods: Patients with past history or symptoms of stroke based on their medical records were excluded. MRI of T1-weighted, T2-weighted, and FLAIR images were performed in 234 CKD patients without dialysis (140 males and 94 females, 61±15.7 years, serum creatinine 2.96±2.62 mg/dl). SCI: SCI was found in 67 CKD patients (28.6%). Patients with SCI were significantly older (p<0.0001) than those without. Systolic, diastolic and pulse pressures were significantly higher (p<0.0001, p=0.0230, and p<0.0001, respectively) in the former than in the latter. Total cholesterol, triglyceride, and LDL-cholesterol were significantly higher (p=0.0221, p=0.0473, and p=0.0305, respectively). Serum creatinine was significantly higher (3.67±2.58 vs. 2.68±2.60 mg/dl, p<0.01), and eGFR was significantly lower (22.3±18.3 vs. 42.1±35.4 ml/min/1.73m², p<0.001). As CKD stage was advanced, prevalence of SCI was significantly increased (p=0.0014, χ²-test). PVH were found in 117 CKD patients (50%). Patients with PVH were significantly older (p<0.0001) than those without. Systolic, diastolic and pulse pressures were significantly higher (p<0.0001, p=0.01011, and p<0.0001, respectively) in the former than in the latter. Total cholesterol, triglyceride, and LDL-cholesterol were significantly higher (p=0.0014, p=0.0183, and p=0.0488, respectively). Serum creatinine was significantly higher (3.80±2.73 vs. 2.12±2.26 mg/dl, p<0.0001), and eGFR was significantly lower (22.3±19.4 vs. 50.9±37.1 ml/min/1.73m², p<0.001). As CKD stage was advanced, prevalence of PVH was significantly increased (p=0.0001, χ²-test). In multivariate logistic regression analyses with adjustment of age, gender, and several confounders, eGFR was an independent, significant factor associated with the prevalence of both SCI and PVH (OR 0.969 [95% CI 0.961-0.991] p=0.001, respectively). There was no significant relationship between SCI/PVH and the presence of diabetes mellitus or dyslipidemia.

Conclusions: Prevalence of cerebral ischemic lesions of SCI and PVH is significantly higher as the CKD stage is advanced. Decreased renal function is an independent, significant factor associated with SCI and PVH, after adjustment of several confounders. Decreased renal function could be associated with future cerebrovascular events.

**OSa003** EFFICACY OF CANAKINUMAB (ACZ885) IN THE PREVENTION OF FLARES IN GOUT PATIENTS INITIATING ALLOPURINOL THERAPY

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Introduction and Aims: Urate lowering therapy (ULT) is recommended for gout patients experiencing frequent flares. However, initiation of ULT is associated with an increase in flares. Many patients with gout have comorbidities such as chronic kidney disease which may limit their treatment options. Recent evidence suggests that gouty inflammation is driven by the production of interleukin-1β (IL-1β). Thus, suggesting that targeted IL-1β blockade may be a valuable prophylaxis option for patients initiating ULT. This study compared the efficacy and safety of canakinumab, a fully human anti-IL-1β antibody vs colchicine for prevention of flares in gout patients initiating allopurinol therapy.

Methods: In this 24-week, multi-center, double-blind, double-dummy study, patients (20-79 years) initiating allopurinol were randomized (1:1:1:1:1:1:2) to receive a single subcutaneous (sc) dose of canakinumab (25, 50, 100, 200 or 300 mg), or canakinumab 150 mg sc administered in 4 divided doses every 4 weeks (50+50+25+25mg [q4wk]) or colchicine 0.5 mg daily for 16 weeks. The primary objective was to determine the canakinumab dose having comparable efficacy to colchicine with respect to the number of gout flares occurring during the first 16 weeks. Secondary objectives included the percentage of patients experiencing flares and the frequency of flares per patient. Herein, we report the results of a pre-planned interim analysis.

Results: 452 patients were randomized; 38 patients (7-11% of each study group) had discontinued the study at the time of the interim analysis. All canakinumab doses were superior to colchicine in preventing flares. Therefore, a canakinumab dose comparable to colchicine could not be estimated. Based on a negative binomial model, all canakinumab groups reduced the event rate significantly compared to colchicine (rate ratio estimates 25 mg, 0.52; 50 mg, 0.34; 100 mg, 0.25; 200 mg, 0.40; 300 mg, 0.52).
immune and inflammatory mechanisms

mg. 0.29, q4wk, 0.38; p<0.05 for all comparisons). The percentage of patients with flares was lower for all canakinumab groups (13.5-23.6%) vs colchicine (45.9%, p<0.05 for all canakinumab doses). The number of flares per patient during the 16-week period was lower for all canakinumab groups vs colchicine (least squares mean, 0.21-0.70 vs 0.74) and reached statistical significance for the canakinumab 100 mg and 300 mg groups (p<0.05). Adverse events (AEs) were reported in 44.4-58.5% for canakinumab vs 51.9% for colchicine. Serious AEs were reported in 2 (3.6%; 25 mg), 2 (3.7%, 50 mg), 2 (3.7%,100 mg), 3 (5.6%, 200 mg), and 1 (1.9%, q4wk) patients receiving canakinumab and in 6 (5.6%) patients receiving colchicine. A fatal myocardial infarction occurred in one patient in the colchicine group.

Conclusions: Canakinumab significantly reduced the risk of flares in gout patients initiating allopurinol therapy compared with colchicine and was well tolerated.

Disclosure: Dr. So reports having received consulting fees or payment for participation in advisory board meetings from Opsona, Novartis, Wyeth and Abbott, having equity interests in Pfizer, and having received lecture fees from Bristol Myers Squibb and grant support from Fonds National Suisse.
Methods: BMP-2 expression in CD133+CD24+Pax-2+ cells was evaluated in vivo by confocal microscopy on adult human renal tissue. ARPCs were isolated through "magnetic cell sorting". On these cells BMP-2 and BMP receptors (BMPRs) gene (RT-PCR) and protein (ELISA/immunoblotting) expression were studied in basal condition and after inflammatory stimuli. Myofibroblastic markers alpha-smooth muscle actin (SMA), collagen-I and fibronectin were evaluated by immunoblotting in ARPCs and proximal tubular cells (HK-2) after BMP-2 stimulation. Intracellular reactive oxygen species (ROS) production was evaluated by 2',7' dichlorodihydrofluorescein. NADPH-dependent superoxide generation was evaluated by chemiluminescence. Nox4 (NADPH oxidase renal isofrom) protein expression was studied by immunoblotting.

Results: BMP-2 was basally expressed in normal adult human kidney with ARPCs being the main source of this growth factor. In vitro, TNF-alpha (200 U/ml) significantly induced both BMP-2 gene expression (1.7±0.2 fold change vs basal, p=0.03) and protein secretion (basal 48h 65±1.0 pg/ml; TNF-alpha 48h 152.5±19.8 pg/ml, p=0.002) in ARPCs. These cells expressed BMPRs (ALK-2, ALK-3, ALK-6), suggesting their potential responsiveness to BMP-2. Incubation of ARPCs with this growth factor (30 ng/ml) significantly enhanced ROS production (basal 36±15.3 AU; BMP-2 15' 65±17.8 AU, p=0.03), NADPH oxidase activity (basal 86±38.2 AU; BMP-2 15' 21±5.4 AU, p=0.01) and Nox4 protein expression (basal 0.3±0.2 AU; BMP-2 5' 1±0.6 AU, p=0.003). BMP-2 incubation for 5 days induced alpha-SMA (basal 0.3±0.1 AU; BMP-2 0.8±0.2 AU, p=0.02), collagen-I (2.4±0.6 fold change vs basal, p=0.03) and fibronectin (1.4±0.1 fold change vs basal, p=0.04) protein expression in ARPCs, but not in HK-2 cells. The oxidative stimulus H2O2 (200 microM) induced alpha-SMA expression in ARPCs (basal 1.0±0.2 AU; H2O2 2.6±0.8 AU, p=0.03), while the anti-oxidant N-acetyl-cysteine inhibited BMP-2-induced alpha-SMA expression (BMP-2 2±0.0 AU; BMP-2+NAC 1.3±0.4 fold change; p=0.04).

Conclusions: We demonstrated for the first time that: 1. ARPCs express BMP-2; 2. this expression is significantly up-regulated by inflammatory stimuli; 3. BMP-2 may induce the commitment of ARPCs toward a myofibroblastic phenotype. 4. This pro-fibrotic effect is mediated by the activation of Nox4. Our findings may suggest a novel mechanism linking AKI with progressive renal damage.

OSa009 IDENTIFICATION OF A NOVEL AUTOIMMUNE PROTEIN USING THE SERUM OF MEMBRANOUS NEPHROPATHY PATIENTS

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Introduction and Aims: Membranous Nephropathy (MN) is the most common cause of nephrotic syndrome in adults and its definitive diagnosis is carried out by biopsy. Thanks also to some lately published data, an autoimmune condition is strongly suspected in MN. Putatively involved proteins, responsible for the idiopathic form of the disease, have been found in a purposeful majority of cases. At least 30% of cases have still to be immunologically defined. The aim of this work is to identify these proteins by screening a lambda-phiage library using patients sera.

Methods: Thirty nephrotic patients affected by MN (15 for the discovery phase and 15 for the confirmation phase) were recruited for this work. Fifteen patients affected by other glomerulonephrites and 15 healthy individuals were also recruited as control subjects. A commercial kidney cDNA phage library was screened using the above described discovery group pooled sera, in order to detect positive signals following antigen-antibody recognition.

Results: We detected one phagemid clone expressing a protein which was shown to be targeted by the antibodies of the pooled MN sera. The cDNA insert carried by the phagemide was subsequently sequenced and identified as Synaptonemal Complex protein 65 (SC65), also known as No55. The pure protein was used to test the autoimmunity of single sera: we found that 6 out of 30 MN sera (30%), only 1 out of 15 amongst non-MN controls (6.7%) and none of the healthy subjects were positive.

Conclusions: Anti-No55 autoantibodies are involved in MN, as we observed through the 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 autoimmune proteins might therefore help revealing new pathogenetic mechanisms of MN, developing new diagnostic methods and, eventually, defining new prognostic guidelines.

OSa007 IL-9 PRODUCTION BY Tregs RECRUITS MAST CELLS THAT ARE ESSENTIAL FOR Treg-INDUCED IMMUNE-SUPPRESSION

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Introduction and Aims: Both, mast cells (MC) and regulatory T cells (Treg) have gained attention as immunosuppressive cell populations, especially in autoimmune diseases.

Methods: To investigate the possible interaction between these populations, we used the well- characterized Th1- and Th17-dependent model of nephrotoxic serum nephritis (NTS), in which both MC and Tregs have been shown individually to play a protective role.

Results: Transfer of wildtype Tregs into wildfiretype recipients almost completely prevents development of NTS and leads to a profound increase of MC in the renal draining lymph nodes (LN). By contrast transfer of wildtype Tregs into animals deficient in MC (Kir/aK+/- mice), which are characterized by an exaggerated susceptibility to NTS, no longer exhibited protective effects. These data underscore the pivotal role played by MC in Treg-mediated immunosuppression. Due to the well-described role for IL-9 in the induction of MC, we evaluated the role of IL-9 as mediator of Treg interaction. Strikingly, injection of blocking mAb to IL-9 into mice receiving Tregs abrogated protection from NTS. Furthermore, transfer of Treg isolated from IL-9 deficient animals also failed to protect from NTS. In the absence of Treg-derived IL-9, MC fail to arise in the tissue, despite the fact that IL-9 deficiency does not alter the general suppressive activity of Treg.

Conclusions: In summary, we provide the first direct in vivo evidence that the neproprotective effects of Treg cells critically depend on IL-9 mediated attraction of MC into kidney-draining LN thereby limiting the tissue-damaging immune response.

OSa008 IDENTIFICATION OF A NOVEL AUTOIMMUNE PROTEIN USING THE SERUM OF MEMBRANOUS NEPHROPATHY PATIENTS

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Introduction and Aims: Dense deposit disease (DDD) or membranoprolif-erative glomerulonephritis type II is a rare kidney disorder that is associated with dysregulation of the alternative pathway of complement. C3 nephritic factor (C3NeF), an autoantibody binding to a neoepitope on the alternative pathway C3 convertase, is commonly detected in patients with DDD. A 6.3-year old patient was diagnosed with DDD, had normal APTh50 value but low C3 level, the latter indicative of complement activation. The complement activation fragments C3d and Ba were detected in plasma. The patient was negative for C3NeF. We hypothesized the presence of other anti-complement autoantibodies.

Methods: Three nephrotic patients affected by MN (15 for the discovery phase and 15 for the confirmation phase) were recruited for this work. Fifteen patients affected by other glomerulonephrites and 15 healthy individuals were also recruited as control subjects. A commercial kidney cDNA phage library was screened using the above described discovery group pooled sera, in order to detect positive signals following antigen-antibody recognition.

Results: We detected one phagemid clone expressing a protein which was shown to be targeted by the antibodies of the pooled MN sera. The cDNA insert carried by the phagemide was subsequently sequenced and identified as Synaptonemal Complex protein 65 (SC65), also known as No55. The pure protein was used to test the autoimmunity of single sera: we found that 6 out of 30 MN sera (30%), only 1 out of 15 amongst non-MN controls (6.7%) and none of the healthy subjects were positive.

Conclusions: Anti-No55 autoantibodies are involved in MN, as we observed through the 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 autoimmune proteins might therefore help revealing new pathogenetic mechanisms of MN, developing new diagnostic methods and, eventually, defining new prognostic guidelines.
Results: An IgG3 antibody that bound to the serine protease factor B was identified in the patient’s plasma. The autoantibody bound within the Bb fragment of factor B, which is part of the alternative complement pathway C3 convertase C3bBb. Purified plasma IgG fraction of this patient showed dose-dependent binding to a solid-phase C3 convertase. Upon binding, the anti-factor B autoantibody stabilized the convertase both against the intrinsic and the extrinsic, factor H-mediated decay, similar to C3NeF. However, in contrast to C3NeF which enhanced lysis, the anti-factor B autoantibody dose-dependently inhibited rabbit erythrocyte lysis. Analysis of the supernatants revealed increased amounts of C3a but decreased levels of C5α, indicating that this anti-factor B autoantibody enhances C3 convertase activity and at the same time blocks C5 convertase activity. Measurement of C5a levels in the patient’s plasma samples showed that not all C5 convertases were inhibited in vivo.

Conclusions: In summary, we describe a novel autoantibody in DDD that binds to native factor B as well as the alternative pathway C3 convertase and, by stabilizing the convertase, it enhances C3 turnover without leading to enhanced C5 consumption and terminal pathway activation. Thus, the anti-factor B autoantibody plays a potentially pathogenic role by contributing to C3 fragment deposition in the glomeruli. The results show that autoantibodies others than C3NeF may play a role in DDD and provide rationale for screening and functional analyses of such antibodies in DDD patients.

OSa010 EXOGENOUS GLYCOSPHINGOLIPID MODULATES THE OUTCOME OF EXPERIMENTAL FOCAL AND SEGMENTAL GLOMERULOSCLEROSIS

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Methods: In our study, we established a model of adriamycin-induced nephrotic syndrome in rats. Adriamycin (ADM) (10 mg/kg) or ADM plus GSL-1 (GSL) (50 mg/ml), a pro-Th1 glycosphingolipid derived from the Sphingomonadaceae family of bacteria. Weight gain and renal function, depicted by albuminuria levels, were measured every three days to follow disease development.

Results: GSL-1 treatment concomitantly with ADM administration inhibited albuminuria (ADM: 60 ± 2.3 mg/mL vs GSL: 37 ± 2.3 mg/mL), a phenomenon associated with suppression of renal fibrosis, mesangial expansion and tubular damage. The PCR analysis of renal tissue revealed that ADM administration induced GATA3 (ADM: 1.6 ± 0.1 vs Ctrl: 0.9 ± 0.1), IL-4 (ADM: 2.5 ± 0.5 vs Ctrl: 1.2 ± 0.1) and IL-13 (3.37 ± 0.8 vs Ctrl: 1.0 ± 0.1) gene expression. In contrast, GSL-1 treatment favored T-bet (GSL: 2.5 ± 0.5 vs ADM: 1.0 ± 0.2) gene expression, in concordance with the suppression of type 2 cytokines mRNA (GATA3: 1.0 ± 0.1; IL-4: 1.2 ± 0.1; IL-13: 1.6 ± 0.1). We next initiated the GSL-1 treatment at day 4 after ADM injection, when proteinuria and albuminuria were already established. The albuminuria in GSL-1 treated mice decreased in a time-dependent fashion, suggesting recovery of renal function. Indeed, the histological analysis at day 28 demonstrated the conservation of renal structure in comparison to ADM mice. Further Q-PCR analysis of renal tissue revealed that ADM administration induced an early expression of TGF-β1 (ADM: 2.6 ± 0.2 vs Ctrl: 1.0 ± 0.1) gene that resulted in latter increase in TIMP-1 (ADM: 10.0 ± 0.1 vs Ctrl: 0.8 ± 0.1) and MMP9 (ADM: 1.6 ± 0.3 vs Ctrl: 1.0 ± 0.1) expression, indicating that a deregulation in the apparatus involved with deposition and degradation of matrix proteins can be part of the mechanisms implicated in ADM-induced FSGS. GSL-1 treatment suppressed MMP9 and TIMP-1 genes (comparable to non-treated mice levels), although it increased TGF-β1 mRNA expression induced by ADM (GSL: 3.9 ± 0.5).

Conclusions: Taken together our data demonstrate that exogenous pro-Th1 glycosphingolipids can be an alternative approach in FSGS management.

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Transplantation – clinical research 1

OSa011 HYPERTENSION FIVE YEARS AFTER DONOR NEPHRECTOMY

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Introduction and Aims: There are concerns about the long term consequences of live kidney donation. We examined renal function, blood pressure and prevalence of hypertension five years post-donation.

Methods: Files were retrieved from all live kidney donors in Norway during 1997-2002. Preoperative characteristics were registered, and five year data were collected from the Norwegian Live Donor Registry.

Results: Five year follow-up data were available in 241 out of 482 donors. Baseline characteristics did not differ between donors with and without follow-up data, except for diastolic blood pressure (lower in the group with follow-up data (76.1 ± 6.8 mmHg vs. 77.6 ± 7.4 mmHg, p = 0.02)). In these 241 donors, mean age was 47.4 ± 11.8 years, mean body mass index (n = 236) 25.0 ± 3.1 kg/m2, mean s-creatinine 81.5 ± 9.7 mmol/l and mean creatinine clearance (n = 239) 112.5 ± 28.2 ml/min. There were 38.6% men, and 37.1% were smokers (n = 221). Mean blood pressure pre-donation was systolic 122.6 ± 10.1 mmHg and diastolic 76.1 ± 6.8 mmHg, increasing to 127.4 ± 15.4 mmHg (p < 0.001) and 78.9 ± 8.2 mmHg (p < 0.001) at follow-up. Seven donors (2.9%) had hypertension before donation. At follow-up 64 donors (26.6%) had hypertension, and 43 of these were in need of antihypertensive medication. Among the seven donors with hypertension before donation, two were now normotensive. Mean s-creatinine at follow-up was 96.6 ± 16.8 mmol/l. Two donors (n = 231) had proteinuria.

Conclusions: Five years after kidney donation 26.6% of donors were diagnosed with hypertension. This considerable proportion of hypertensive donors justifies mandatory long term follow-up and the use of donor registries.

OSa012 A SIGNIFICANT INCREASE IN ONE YEAR POST-TRANSPLANT RENAL ARTERIAL INDEX PREDICTS GRAFT LOSS

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Introduction and Aims: Conflicting data have been reported concerning the use of kidney graft arterial resistance index (RI) measured by Doppler...
to predict graft loss. We hypothesized that changes in RI values could carry better information than a single measure of RI.

**Methods:** Two hundred and seventy four renal transplant recipients were included in the study. We tested whether changes in renal arterial resistance index between four and twelve months post-transplant (DRI 4-12) were predictive of graft loss.

**Results:** Neither four months nor one year RI predicted graft loss. The area under the ROC curve of DRI 4-12 for graft loss was 0.71. A DRI 4-12 ≥ 10% had the best sensitivity and specificity. One year post-transplant, 21% of the study population had DRI 4-12 ≥ 10%. Forty two patients (15.3%) experienced graft loss during follow-up. The incidence of graft loss was higher in patients with DRI 4-12 ≥ 10% (3.4% vs 1.3%; p=0.009).

In multivariate analysis, patients with DRI 4-12 ≥ 10% had an increased risk of graft loss (HR 7.51 [95% CI: 1.87-30.21], p=0.005).

**Conclusions:** A variation in RI ≥ 10% in the first year post-transplant is an independent risk factor for graft loss in RTRs.

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**OSa013**

**TWENTY YEARS OF NATURAL HISTORY OF HEPATITIS C VIRUS INFECTION IN RENAL GRAFT RECIPIENTS**

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**Introduction and Aims:** In 1989, 541 patients with a functioning kidney, transplanted between 1972 and 1989, were tested for hepatitis C virus (HCV) infection. Seventy six patients were excluded because of hepatitis B virus infection. In order to study the natural history of HCV infection in renal graft recipients, we reported our single-center experience of 465 HbsAg positive patients.

**Methods:** In 1989 HCVRNA replication was studied by qualitative (AmpliCor Roche) and quantitative (AmpliCor Monitor HCV Roche/PCR) methods as well as light chain (IgG-ELISA). The genotype was determined by using the sequence-based typing method.

**Results:** Of the 465 HCV+ recipients, 37% had a long-term survival. HCV+ patients had more frequent proteinuria (p=0.001), follow-up characteristics: HCV+pts had more frequent proteinuria (>1g/day) than HCV- (p=0.001), the percentage of rejection episodes was similar Post enrollment patient survival was 90%, 75%, 58% and 47% at 5, 10, 15 and 20 years after transplantation (p<0.001). Cox proportional hazards multivariate analysis identified age at transplantation (stratified according age in 2 groups <40 and ≥40 years) (p=0.001) and HCV (p=0.019) as a predictor of patient mortality. Causes of death of the 138 pts (79HCV+ vs 59-) were cardiovascular in 50% (34+vs35-), neoplastic 22% (15+vs15-), sepsis in 13% (8+vs9-), liver failure in 12% (16+vs6-), hepatocarcinoma in 4% (6+). Graft survival was 76%, 54%, 32% and 18% in HCV+ and 84%, 67, 50% and 37% in HCV- at 5,10,15 and 20years, respectively (p=0.001). Hepatocarcinoma occurred in 6 AZA treated pts after of 23+6 years.

**Conclusions:** The presence of HCV+ in the long-term significantly reduces patient and graft survival rates, even in the younger pts group. However, in HCV+ pts causes of death were extrarenal in73% and 27% related to liver failure. 43% of HCV+ pts died of a result of cardiovascular events, the leading cause of death in renal tx pts. Overall, in our single center experience, after 20 years from enrollment, approximately 18% of our HCV+ and 37% of our HCV- patients have a long-term survival.

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**OSa014**

**CIRCULATING ANGIOPOIETIN-2 LEVELS PREDICT MORTALITY IN KIDNEY TRANSPLANT RECIPIENTS: A PROSPECTIVE CASE-CONTROL STUDY**

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**Introduction and Aims:** Endothelial activation and dysfunction represent early events in the pathogenesis of arteriosclerosis. Angiopoietin 2 (Angpt2) impairs endothelial function by preventing Ang-1 from binding to their common endothelial-specific receptor Tie2. We have recently shown that Angpt2 is increased in essential hypertension, increases with the progression of chronic kidney disease and correlates with arteriosclerotic burden in dialysis patients. Here we examined whether circulating Angpt2 levels also predict mortality and/or graft-loss in renal transplant recipients.

**Methods:** For this prospective case control study we selected 130 kidney transplant recipients who died (n=60) or returned to dialysis (n=70), as well as 130 age and gender-matched kidney transplant recipients without an event (controls) from a total of 993 kidney transplant recipients followed prospectively at a single transplant center for a median of 2.3 years. Serum Angpt2 at baseline was measured by in-house immuno-luminometric assay. Association of baseline Angpt2 levels with all-cause mortality and graft failure was examined in competing risk regression analyses.

**Results:** Median Angpt2 concentrations were significantly higher in patients who died and/or returned to dialysis (median interquartile range = IQR) 4.0 [2.5-6.0] ng/mL as compared to patients who did not suffer from any event during the study period (2.8 [2.0-3.7] ng/mL; P < 0.001). Ln (natural log) Angpt2 levels correlated positively with C-reactive protein levels (r=0.380, P < 0.001), and the Charlson Comorbidity Index (r=0.213, P < 0.001), and were inversely associated with eGFR (r= -0.276, P < 0.001) and serum albumin concentrations (r=-0.372, P < 0.001). In multivariate analyses baseline Angpt2 levels independently predicted mortality (multivariable adjusted hazard ratio associated with one log unit higher Ang2 level: 2.30 (95% confidence interval: 1.20-4.40) p=0.012). Angpt2 levels were not associated with graft loss (p=0.17).

**Conclusions:** Angpt2 is elevated in kidney transplant recipients, particularly those with higher co-morbidities and micro-inflammation, possibly reflecting pronounced endothelial activation and dysfunction. Circulating Angpt2 is also a strong and independent predictor of mortality in stable kidney transplant recipients.
**Acute kidney injury – basic research**

**OSa017**

**PRE-CONDITIONING AUGMENTS MESENCHYAL STEM CELLS SURVIVAL AND ENGRAFTMENT IN RENAL ISCHEMIA REPERFUSION**

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**Introduction and Aims:** Ischemia reperfusion injury is the major cause of acute renal failure (ARF). Treatment options for this poor prognostic disease include dialysis and the kidney transplant but certain limitations have restricted these from becoming practical solutions. Mesenchymal stem cells (MSCs) are diverse cell type having multilineage differentiation potential. The success of MSCs engraftment depends on their prolonged existence in the target tissue. To improve the outcome of the cell based therapy, the strategies for their enhanced survival is very important. This study is aimed to enhance the survival, engraftment of MSCs in ischemic kidney by S nitroso N acetyl penicillamine (SNAP, a nitric oxide donor) pre-conditioning (PC).

**Methods:** MSCs were grown in IMDM and incubated for 6 hrs with DMSO used as solvent for SNAP (control group), with 100 μM SNAP (SNAP group), 100 μM SNAP and 1 μM methylene blue (MB) that is a nitric oxide synthase (NOS) and guanylate cyclase inhibitor (SNAP + MB group) and 1 M MB (MB group). After 6 hrs MSCs were exposed to 200 M H2O2 for 1 hr to find the effects of these treatments on cell injury, viability and proliferation. For in vivo study, in vitro treated MSCs from all above-mentioned groups were transplanted in rat renal ischemia reperfusion injury model in relevant groups (n=6 in each group) with an additional ischemic group as control injected with medium only. Kidney function, fibrosis, cyto-protective genes expression, MSCs homing and engraftment were assessed after 3 weeks.

**Results:** Improved cell viability, reduced cellular injury and apoptosis was detected after SNAP pre-conditioning. These effects were diminished in SNAP + MB and MB (1μM) pre-treatment of MSCs. SNAP PC, in MSCs up-regulated the expression of insulin like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), Akt, stromal derived factor-1 (SDF-1) and BCL-2 as compared to other treatment groups. SNAP PC induced up-regulation of proliferating cell nuclear antigen (PCNA) gene expression showing enhanced proliferation in MSCs. These findings corroborated to in vivo data which demonstrated that transplantation of SNAP pre-conditioned MSCs in rat renal ischemic model improved renal function (creatinine clearance and BUN) in a temporal fashion. The histological findings showed decreased collagen deposition in SNAP-treated MSCs transplanted group in comparison to all other groups. Homing of transplanted MSCs was also increased in SNAP pre-conditioned group compared to all other groups. Expression of VEGF and endothelial NO was increased in SNAP pre-conditioned group while inducible NOS expression was decreased. Increased number of CM-Dil labeled cells was found to be co-localized with a kidney specific tubular epithelial marker aquaporin-1 suggesting renal tubular cells derived from transplanted MSCs. There was robust increase in Ki67 expression in renal tubular cells in SNAP pre-conditioned group compared to others.

**Conclusions:** SNAP PC might be useful to enhance the therapeutic potential of MSCs to attenuate the renal ischemia reperfusion injury.
OSa018 ESTATERS OF HYALURONIC AND BUTYRIC ACID PROTECT RENAL CELLS IN AN IN VITRO MODEL OF ISCHEMIC INJURY

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Introduction and Aims: Acute kidney injury (AKI) represents a common clinical condition still associated with high mortality and morbidity despite significant advances in supportive care. Recently we demonstrated the improvement of renal function after ischemic damage, by the use of human mesenchymal stem cells isolated from term placentas. This effect, probably due to the secretion of paracrine factors, is remarkably higher when the cells are pretreated with esters of Hyaluronic and Butyric acids (HB). Here we investigate if the use of HB could also protect kidney cells from death due to oxidative stress in an in vitro model of ischemic injury. Methods: Kidneys from two-day-old newborn rats were removed aseptically, minced and digested by collagenase. The released rat kidney cells (rKCs) were cultured in RPMI 1640 with 10% fetal bovine serum. Oxidative stress was induced by H2O2 to simulate in vitro ischemia/reperfusion damage. rKCs were treated for 6-16-24h with different concentrations of H2O2 (25-50-100-200-400μM) to evaluate the mode of death (apoptotic vs necrotic). In subsequent experiments, cells were pretreated with HB (1g/l) or grown in culture media for 24h, before adding H2O2 (25-50-100μM) for 6-16-24h. Cell death was measured by quantification of lactate dehydrogenase (LDH) released into the media, as marker of necrosis, and analysis of caspase-3 activity by fluorometric assay, to evaluate apoptosis. To confirm the mode of cell death, we analyzed the cleavage of procaspase-3 by Western Blot. Results: A dose-dependent activation of caspase-3 was observed at concentrations of H2O2 lower than 100μM. Higher doses caused necrosis as confirmed by the release of LDH into the supernatant. The treatment with HB strongly inhibits cell death after oxidative damage. This effect is significant after 16h of treatment with H2O2 50-100μM (P<0.05) and peaked at 24h, when the mortality rate of rKCs pretreated with the ester is 38% lower than non pretreated cells. Western Blot analysis shown that H2O2 treatment caused marked reduction of procaspase-3 expression, indicating that this protein has been cleaved and consequently activated to caspase-3. This effect is dramatically reduced by the treatment with HB. Fluorometric assay confirmed a reduction of caspase-3 activity in cells pretreated with the ester, that after 24h of treatment with H2O2 50-100μM is 46% lower than non pretreated cells. Conclusions: We demonstrated that the pretreatment of kidney cells with HB protect from injuries induced by oxidative stress, like those observed in ischemia/reperfusion. As the timing for cell expansion will involve a substantial delay in stem cell transplanation with respect to the acute phase of AKI, this effect would be important in order to use HB as first aid to rescue a damaged kidney. This intervention may be followed by delayed transplantation of stem cells, eventually preconditioned ex vivo with the same molecule, to enhance a long-term potential for kidney repair.

OSa019 HEME OXYGENASE-1 IS INVOLVED IN KIDNEY PROTECTION FROM ISCHEMIA AND REPERFUSION INJURY BY MODULATING ACTIVATION OF IMMUNE CELLS

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Introduction and Aims: Heme oxygenase (HO)-1 is an enzyme able to convert the molecule heme to biliverdin and releasing other products such as carbon monoxide and free iron. This enzyme has been associated to renal function regulation and protection during inflammatory diseases. Ischemia and reperfusion injury (IRI) is an acute inflammatory response considered to be the main cause of acute renal injury in kidneys. Dendritic cells (DC) and T cells have been recently associated to have a role in IRI. We investigated if mice treated with heme, a HO-1 inducer, were protected form IRI and the possible changes in T cells and DC in this model. Methods: We treated C57Bl/6 mice with 25 mg/kg of hemin 24h before surgery. We used sham treated and non-treated control groups. One day after surgery, we collected renal lymph nodes and kidneys and we analyzed cells and cytokines by FACS, quantitative PCR and bioplex. The renal function was evaluated by urea and creatinine levels in the serum. Results: After 24h of IR we observed that heme treated animals were protected from IRI presenting lower levels (85±12mg/dl) of urea comparing to control (212±18mg/dl). The same difference was observed measuring the creatinine. The heme group presented two-fold the levels of HO-1 by real-time PCR, corroborating with the idea that this enzyme is involved in renal protection in inflammatory insults. We also measured expression and levels of pro-inflammatory cytokines, which were decreased in heme group. In order to investigate the influence of immune cells in IRI, we checked the phenotype of DC and T cells and we observed that DC from heme expressed higher levels of CD80, CD86 and MHC class II then control group. On the other hand, the population of activated T cells (CD4*CD69*) was decreased in heme treated animals. Conclusions: We concluded that heme is involved in IRI protection, which is probably due to an increase of HO-1 expression. The increase of HO-1 possibly influences changes in DC and T cells phenotype during IRI providing a less inflammatory environment in the kidney and highlighting the HO-1 as a therapy alternative. FINANCIAL SUPPORT: FAPESP and CNPq.

OSa020 DELETION OF VHl IN THE THICK ASCENDING LIMB PROTECTS FROM ISCHEMIC ACUTE KIDNEY INJURY

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Introduction and Aims: Ischemia is a central pathophysiological factor in the development of acute kidney injury (AKI). In marine models of AKI, like in ischemia/reperfusion experiments, tubular ischemic necrosis and activation of the hypoxia-inducible factors (HIF) occur in particular in the outer medulla of the kidney involving the proximal tubule and the thick ascending limb (TAL). We therefore investigated the effects of genetic HIF induction in the TAL in ischemia/reperfusion experiments. Methods: To target the TAL the Tamm-Horsfall protein (THP) promoter was used to drive Cre recombinase expression in transgenic mice which were then crossed with mice carrying floxed von Hippel-Lindau (VHL) gene. Ischemic AKI was induced by clamping both renal pedicles for 25 min, with 3 days of reperfusion afterwards. Results: TAL specific knockout of the VHL gene led to stable expression of HIF1alpha exclusively. No further HIF induction was seen in other organs and no erythrocytosis was noted. HIF1 accumulation in the TAL was associated with cellular target gene induction (e. g. Glucose transporter-1). Clamping of the renal pedicles led to profound ischemic injury in the outer medulla. Compared to wildtype mice, VHL knockouts significantly had better histological tubular necrosis scores, lower creatinine values and recovered more quickly from AKI. Conclusions: VHl deletion in the TAL functionally and morphologically protected mice from ischemic AKI. Our findings underscore the role of the TAL in the pathophysiology of AKI.
GREMLIN, A BMP-7 ANTAGONIST, INDUCES EPITHELIAL MESENCHYMAL TRANSITION THROUGH THE SMAD SIGNALING

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Introduction and Aims: Epithelial mesenchymal transition (EMT) is an important process that contributes to renal fibrosis, being TGF-β/Smad a key EMT signaling pathway. It has been proposed that the BMP-7 antagonist, gremlin, could participate in EMT as a downstream mediator of TGF-β. The aim of this work was to examine the presence of gremlin and its correlation with fibrosis and EMT in a broad group of human nephropathies and to define if gremlin could directly modulate EMT in cultured renal cells, evaluating the involvement of the TGF-β/Smad pathway in this process.

Methods: In human renal biopsies the expression of gremlin, epithelial and mesenchymal markers were studied by in situ hybridization and immunohistochemistry. Human tubular epithelial cells (HK-2 cell line) were stimulated with recombinant gremlin (human and murine). EMT was evaluated by de novo protein expression of a-SMA and Vimentin (Western blotting, immunocytochemistry and confocal microscopy) as well as the expression of the epithelial markers E-Cadherin and Cytokeratin.

To investigate whether gremlin regulates Smad-mediated gene expression, cells were co-transfected with a gremlin expression vector (GREM-GFP) and luciferase Smad reporter plasmid. To block Smad pathway activation cells were transfected with Pc-DNA3-FLAG-Smad7.

Results: In several progressive human diabetic and non-diabetic glomerular diseases, gremlin was highly expressed in areas of tubulo-interstitial fibrosis, strongly colocalized with tubular TGF-β, Smad activation and EMT markers. Stimulation of human tubular epithelial cells (HK2 cell line) with a gremlin expression vector also caused EMT phenotype changes. Gremlin increased TGF-β mRNA and protein release in HK2 cells. The blockade of TGF-β by a neutralizing antibody against active TGF-β, diminished gremlin-induced EMT changes. To elucidate the intracellular mechanisms activated by gremlin the Smad pathway was investigated. Gremlin caused a rapid activation of Smad signaling (Smad2/3 phosphorylation) and EMT markers, de novo protein expression of mesenchymal markers, such as a-smooth muscle actin and vimentin, and increased fibronectin release. Transfection of HK2 cells with a gremlin expression vector also caused EMT phenotype changes. Gremlin increased TGF-β mRNA and protein release in HK2 cells. The blockade of TGF-β by a neutralizing antibody against active TGF-β, diminished gremlin-induced EMT changes. To elucidate the intracellular mechanisms activated by gremlin the Smad pathway was investigated. Gremlin caused a rapid activation of Smad signaling (Smad2/3 phosphorylation and nuclear translocation) in tubuloepithelial cells. Smad7 overexpression, which blocks Smad2/3 activation, diminished Smad-dependent gene transcription and EMT changes in gremlin-transfected tubuloepithelial cells.

Conclusions: We propose that gremlin is involved in renal fibrosis by inducing EMT through TGF-β/Smad signaling.
represent a link between the increased vascular responsiveness and tissue damage.

**OSa024** GENE REGULATION BY microRNA IS ASSOCIATED WITH CD8+ T CELLS IMMUNE DEVIATION IN RENAL CELL CARCINOMA PATIENTS: ROLE OF JAK3 AND MCL-1

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**Introduction and Aims:** Mammalian microRNAs (miRNAs) are important regulators of gene expression and numerous miRNAs are expressed abnormally and correlate with tumorigenesis, progression, and prognosis of various haematological and solid tumours. In patients with renal cell carcinoma (RCC), T cell immune dysfunctions have been described, which is consistent with their inability to mediate clinically beneficial outcomes in vivo. In the present study, we aimed to assess gene expression profiles and their regulatory mechanisms by miRNAs, on CD8+ T cells from RCC patients, at basal (day 0) and after stimulation against RCC line (day 35).

**Methods:** We compared autologous and allogeneic CD8+ T-cell responses against RCC line generated from RCC patients and their HLA-matched healthy donors, using mixed lymphocyte/tumor cell cultures (MLT). The expansion of tumor-specific cytotoxic lymphocytes allowed us to analyze the gene expression profiles of CD8+ T cells by microarray approach and then identify molecular mechanisms of gene regulation by miRNAs analysis.

**Results:** Comparison of gene expression data, using microarray, in allogeneic CD8+ T-cell responses against RCC line generated from RCC patients and their HLA-matched healthy donors, using mixed lymphocyte/tumor cell cultures (MLT). The expansion of tumor-specific cytotoxic lymphocytes allowed us to analyze the gene expression profiles of CD8+ T cells by microarray approach and then identify molecular mechanisms of gene regulation by miRNAs analysis.

**Conclusions:** Our results indicate that miR-29b and miR-198 play a key role in regulating immune-mediated mechanisms by interfering in CD8+ T cells gene expression of JAK3 and MCL-1 and may have important implications for therapeutic strategies in RCC.

**OSa025** INCREASED TRANSIENT RECEPTOR CANONICAL TRPC5 CHANNEL EXPRESSION MIGHT BE RESPONSIBLE FOR HYPERTENSION DURING ERYTHROPOIETIN THERAPY

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**Introduction and Aims:** Previous studies indicated that hypertension and stroke are major side effect of erythropoietin therapy in patients with chronic kidney disease. Transient receptor potential canonical (TRPC) channel expression has been associated with elevated blood pressure.

**Methods:** In the present study we investigated whether erythropoietin (EPO, final concentration 1330 mIU/mL) affects TRPC channel expression in cultured endothelial cells. TRPC expression was investigated using quantitative RT-PCR and Western blotting in human endothelial-derived cell line (EAhy926). Membrane-permeable 1-oleoyl-2-acetyl-sn-glycerol (OAG), final concentration 100 μmol/L) induced calcium influx in Fluo-4-loaded EAhy926 cells was investigated using confocal laser scanning microscopy.

**Results:** The administration of EPO significantly increased TRPC5 transcripts in endothelial cells from 0.19±0.03 to 0.23±0.01 arbitrary units (each n=5; p<0.05), whereas the expression of TRPC3 was not significantly affected (0.19±0.03 vs. 0.15±0.01). In the presence of the phosphoinositide-3-kinase (PI3)- inhibitor, LY294002 the expression of TRPC5 was further enhanced to 0.45±0.03 (n=5; p<0.05). Western blotting confirmed that EPO increased TRPC5 channel protein expression in EAhy926 cells. Furthermore, OAG-induced calcium influx was also enhanced in EPO-treated EAhy926 cells by 78% (n=5; p<0.05) compared to the control condition. In the presence of membrane-permeable TRPC blockers, 2- aminoethoxydiphenylborane (2-APB; final concentration 10 μmol/L) or 1-[(3-[4-(methoxyphenyl) propoxy]-3-methoxyphenyl)-1H-imidazole (SKF-96365; final concentration 10 μmol/L), OAG-induced calcium influx was significantly reduced by 55% (p<0.05).

**Conclusions:** These data indicate that erythropoietin increases TRPC5 mRNA, TRPC5 channel protein expression and TRPC5-mediated calcium influx in cultured endothelial cells supporting increased blood pressure and stroke.

**Epidemiology and outcome research 1**

**OSa026** RANDOMIZED CONTROLLED TRIAL OF 1-DEAMINO 8-D-ARGININE VASOPRESSIN IN PERCUTANEOUS ULTRASOUND-GUIDED RENAL BIOPSY

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**Introduction and Aims:** Percutaneous renal biopsy is not a risk free procedure. Bleeding complications still occur in about 1/3 of biopsies and increase hospital stay and costs. We evaluated the effect of pre-biopsy treatment with 1-deamino-8-D-arginine (DDAVP) on the incidence of post-biopsy bleeding complications.

**Methods:** This is a IV phase single centre, double blind, randomized controlled study in patients undergoing ultrasound-guided percutaneous renal biopsy. Patients >16 and < 80 years old, with serum creatinine ≤ 1.5 mg/dL and/or glomerular filtration rate (GFR) > 60 ml/min, blood pressure (BP) <140/90 mmHg and normal coagulation parameters were centrally randomized to receive either DDAVP 0.3 mcg/kg s.c. or 4 ml of saline solution s.c., 1 hour prior to biopsy. The primary outcome was the incidence of post-biopsy hematoma, the secondary outcomes were hemoglobin levels, coagulation parameters, glomerular filtration rate, blood pressure, mean hospital stay and safety of the drug. Post-biopsy bleeding was detected after 24 hours by renal ultrasound. Analysis was conducted by intention to treat.

**Results:** From August 2008 to December 2009 a total of 162 patients, undergoing renal biopsy in our Renal Unit, were enrolled; 80 were allocated to DDAVP and 82 to placebo group. DDAVP compared to placebo significantly reduced the risk of post-biopsy bleeding [980 (11%) versus 25/82 (31%); p=0.01] and size of hematoma 262.7±148.9 mm² versus 409.7±212.8 mm²; p=0.04). At multivariate analysis DDAVP administration was independently associated with a significant reduction of bleeding complications (RR 0.45, CI 95% 0.24-0.85; p=0.01).

**Conclusions:** Pre-biopsy administration of DDAVP significantly reduces the risk of bleeding and size of hematomas in patients undergoing renal biopsy.

**OSa027** C-REACTIVE PROTEIN AND INTERLEUKIN-6 MODIFY THE RISK OF HIGH LEVELS OF ASYMMETRIC DIMETHYL-ARGININE (ADMA) FOR DEATH AND CARDIOVASCULAR EVENTS IN END STAGE RENAL DISEASES PATIENTS

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**Introduction and Aims:** Endothelial dysfunction as assessed by asymmetric
dimethyl-arginine (ADMA) and inflammation been consistently linked to atherosclerosis, death and cardiovascular (CV) events in patients with end stage renal diseases (ESRD). Inflammation amplifies the effect of ADMA on the severity of carotid atherosclerosis in ESRD patients (JASN 15: 490-496, 2002) but it is still unknown whether inflammation and ADMA interact in the high risk of death and CV events in this population.

Methods: In a cohort of 222 haemodialysis patients we investigated the interaction between inflammatory biomarkers (CRP and IL-6) and ADMA levels as predictors of all cause mortality and CV events (average follow-up: 42 months). The effect modification of CRP and IL-6 on the relationship between ADMA and study outcomes (additive model) was analysed by dividing the study population according to the corresponding median values of these biomarkers.

Results: Circulating levels of ADMA (median: 2.4 μM/L, inter-quartile range: 1.6-3.8 μM/L), CRP (7.4 μg/L, 3.4-16.4 μg/L) and IL-6 (5.0 pg/mL, 2.8-9.2 pg/mL) were directly and significantly inter-related (r ranging from 0.13 to 0.33, P<0.05). During the follow-up, 110 patients died and 101 had fatal and non fatal CV events. On univariate Cox regression analysis, patients with both ADMA and CRP above the median values had a relative risk of death [hazard ratio (HR): 2.29, 95% confidence interval (CI): 1.39-3.79] higher than that of patients with ADMA (HR: 1.38, 95% CI: 0.79-2.41) or CRP (HR: 0.94, 95% CI: 0.50-1.75) above the corresponding median value and of those with both ADMA and CRP below these thresholds (reference group) (P for trend=0.001). Similarly, patients with both ADMA and CRP above the median values had a crude relative risk of cardiovascular events [HR: 2.33, 95% CI: 1.49-3.63] higher than that of patients with ADMA (HR: 1.57, 95% CI: 0.96-2.57) or CRP (HR: 1.18, 95% CI: 0.69-2.01) above the median values and of those with both ADMA and CRP below these cut-offs (reference group) (P for trend=0.003). Multivariate models including traditional and not traditional risk factors confirmed a significant ADMA-CRP interaction in predicting both all cause (P for trend=0.01) and CV events (P for trend=0.015). Moreover, the same analyses carried-out for the ADMA-IL-6 interaction showed that patients with both ADMA and IL-6 above the median values were those with the highest relative risk of death and CV events on both univariate [P<0.01] and multivariate analyses [P<0.01].

Conclusions: These data support the hypothesis that inflammation amplifies the ADMA associated risk of death and CV events in ESRD patients on haemodialysis. These analyses further emphasize the need of interventional studies to clarify the nature (causal/non causal) of these relationships.
retained. Finally, severe ED was associated with age (OR 1.11, 95% CI 1.08 to 1.13), presence of nephropathy (OR 2.28, 95% CI 1.43 to 3.63) and CES-D score (OR 4.46, 95% CI 2.31 to 8.61). Being married was found to reduce the risk of severe ED (OR 0.35, 95% CI 0.20 to 0.62) as compared to unmarried patients.

Conclusions: ED is a very common and under-recognised condition in patients on haemodialysis. Mild and moderate ED are probably related to psychological and sociodemographic variables, while severe ED is associated with the severity of kidney disease. There is an unmet need for appropriate diagnosis and management of this highly prevalent condition.

**OSa030 NON-HDL CHOLESTEROL AND RISK FOR NEW ONSET OF VASCULAR EVENTS IN HAEMODIALYSIS PATIENTS**

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Introduction and Aims: Haemodialysis patients show reverse epidemiology between mortality and total cholesterol (TC), although some sub-fractions of lipoproteins are shown to be positively associated with atherosclerosis in renal and non-renal populations. Currently, it is unknown whether dyslipidaemia predicts risk of new vascular events in dialysis patients. We examined the predictive powers of TC, triglyceride (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and Non-HDL cholesterol (Non-HDL) for occurrence of new vascular events in haemodialysis patients.

Methods: This is a retrospective observational cohort study using the data from The Japan Renal Data Registry (JRDR-09108). From 212,632 of the total subjects, we selected 45,390 hemodialysis patients who had had no events in haemodialysis patients. Mild and moderate ED are probably related to age, sex, dialysis vintage and diabetes mellitus; model C, adjusted for the four variables plus BMI, serum albumin, and CRP.

Results: TC showed inverse association with all cause mortality in models A and B, although the association was no longer significant in model C. In contrast, even in model C, risk of MI was positively associated with TC (OR of Q4 vs. Q1, 1.54), TG (OR 1.36), LDL (OR 1.87) and Non-HDL (OR 1.98), and inversely with HDL (OR 0.59). Risk of CI was positively associated with TG (OR 1.30) and Non-HDL (1.28) in model C. CB was not significantly associated with these lipid parameters.

Conclusions: Protein-energy wasting, rather than a low TC itself, explains the reverse epidemiology between TC and mortality. In haemodialysis patients without pre-existing cardiovascular disease, dyslipidaemia was an independent predictor for occurrence of new MI and CI. Particularly, an elevated Non-HDL level was predictive of both MI and CI.

**OSa031 THE RELATIVE RISK OF COMMENCING RENAL REPLACEMENT THERAPY (RRT) WITH INCREASING SOCIAL DEPRIVATION IN THE UK**

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Introduction and Aims: Social and economic deprivation are known to be associated with earlier mortality and end-stage renal disease (ESRD). Area and individual level deprivation have also been independently associated with higher rates of cardiovascular disease and type 2 diabetes. This study aims to quantify the relative risk of commencing RRT with increasing area level socio-economic deprivation, using data from all four countries of the United Kingdom for the first time.

Methods: Age, gender and ethnic origin of the UK general population was obtained from 2001 Census (Office National Statistics). The incident RRT population in 2007 was identified from UK Renal Registry database. The Townsend score was used as a measure of area level socio-economic deprivation; this comprises 4 elements available from the Census: non-ownerships, overcrowded housing, non-car ownership and unemployment. A Townsend score was allocated to the general population and to each incident RRT patient according to their postcode. The general population scores were then divided into quintiles and the patient population assigned to one of these quintiles according to their position in the general population distribution of scores. A relative proportions method was used to calculate the relative risk of commencing RRT, 95% confidence intervals were calculated and Chi squared test for trend was used to identify differences between the quintiles.

Results: There were 6315 incident RRT patients in 2007, of which 2703 had complete data for ethnicity and primary renal disease. Patients excluded due to missing data were similar in terms of age, gender, deprivation scores, and primary renal disease. Forty-seven percent of the study population were over 65 years (of those over 65 years 16% were in the most affluent quintile and 25% in the most deprived quintile, p trend <0.0001). Thirty-six percent had a diagnosis of diabetes, hypertension or reno-vascular disease as the cause for their renal failure (of those 13% were in the most affluent quintile and 27% were in most deprived quintile, p trend <0.0001). Twenty percent of the study population were non-White (of those 8% were in most affluent quintile and 40% were in most deprived quintile, p trend <0.0001).

The unadjusted relative risk of commencing RRT if you live in the most deprived areas was 1.53 (1.38-1.70) compared to the most affluent areas. After adjustment for age and gender this reduced to 1.48 (1.29-1.70) and after adjustment for ethnicity the risk was not significant (1.03 (0.98-1.21).

Conclusions: The risk of commencing RRT in the UK is greatest in the most socially deprived areas, but this increased risk appears entirely explained by the age, gender and ethnic composition of these areas. These factors should be taken into account in the strategic planning of future renal service provision, though it must be remembered that rates of RRT are not synonymous with rates of ESRD.
addition, using a cell culture model (MDCK cells) we confirmed previous findings that ZO-1 knockdown selectively increases the permeability of a low capacity pathway for larger molecules such as mannitol. We demonstrate that simultaneous unidirectional transport of oxalate and mannitol varied in parallel when paracellular permeability was increased by knockdown of ZO-1. In contrast, induction of claudin-10a increased the anion permeability of the tight junction as shown by the change in dilution potential but had no effect on oxalate flux suggesting that oxalate must not traverse this claudin-dependent pathway.

Conclusions: Our studies in mouse intestine confirmed the role of SLC26A6 in mediating transcellular oxalate secretion but indicated that in the absence of SLC26A6 transepithelial oxalate absorption is passive and paracellular. Using a cell culture model with altered paracellular permeability of tight junction proteins we were able to demonstrate that oxalate traverses a low capacity pathway for larger solutes but not the higher capacity claudin-based pores for small solutes. We hypothesize that in the intestine the role of SLC26A6 in mediating active oxalate secretion is to counteract the passive paracellular absorption of ingested oxalate.

OSa033 URIDINE ADENOSINE TETRAPHOSPHATE ACTS AS AN AUTOCRINE HORMONE AFFECTING GLOMERULAR FILTRATION RATE
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Introduction and Aims: Recently, uridine adenine tetraphosphate (Up4A) was described as a strong vasoconstrictor released from endothelial cells after stimulation with mechanical stress. In this study, we isolated and identified Up4A from kidney tissue, and we characterized the essential varying effects of Up4A on the afferent and efferent arterioles.

Methods: Porcine and human kidney tissue was fractionated by size-exclusion-chromatography affinity-chromatography, anion-exchange-chromatography, and reverse phase-chromatography In fractions purified to homogeneity, Up4A was identified by matrix assisted laser desorption/ionisation mass-spectrometry (MALDI-TOF-MS), MALDI-LIFT-fragment-massspectrometry (MALDI-TOF-MALDI-TOF-MS), retention-time comparison, and enzymatic cleavage analysis. We analysed the release of Up4A from cultivated renal proximal tubule cells after stimulation of protein kinase C with OAG. Up4A was identified in renal tissue, and the effect of Up4A on the vascular tone of isolated perfused afferent and efferent arterioles was tested.

Results: Stimulation of tubule cells with OAG increased the release rate of Up4A from tubule cells about ten fold. Up4A acts as a strong vasoconstrictive mediator on afferent arterioles, but has no significant effect on the release of efferent arterioles, suggesting a functional role of Up4A on the afferent arteriole. Because of the predominant effect of the Up4A on the afferent arterioles, we assume that Up4A may decrease glomerular perfusion. Because of the predominant effect of the Up4A on the afferent arterioles, we assume that Up4A may decrease glomerular perfusion, intraglomerular pressure, and hence glomerular filtration rate. The release of Up4A from renal tubular cells may be an additional mechanism whereby tubular cells could affect renal perfusion. Up4A release may further contribute to renal vascular autoregulation mechanisms.

Conclusions: As Up4A occurs in renal tissue and has marked effects on afferent but not efferent arterioles, Up4A may play a role in renal hemodynamics and blood pressure regulation.

OSa034 ★ NOSTRIN PARTICIPATES IN THE ASSEMBLY OF JUNCTIONAL COMPLEXES IN ENDOTHELIAL AND EPITHELIAL CELLS
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Introduction and Aims: We recently compared gene expression profiles of the microcapillaries of stroke-prone and stroke-resistant hypertensive rats (SHRSP vs. SHR) and identified a differentially expressed DNA as the rat homologue of human Nostrin. Nostrin was originally described as endothelial adaptor protein translocating endothelial NO synthase from the plasma membrane by forming a ternary complex with eNOS and Caveolin-1. However, Immunostainings of the kidney revealed Nostrin staining of both endothelial and epithelial cells. Therefore, the aim of the present study was to characterize the role of Nostrin in more detail.

Methods: Expression and localization of Nostrin in different organs and in cultured endothelial and epithelial cells were analyzed using real-time qPCR, western blots and immunohisto- or immunocytochemistry. Moreover, co-immunoprecipitation, pull-down assays as well as siRNA-mediated knock-down of Nostrin were performed to further characterize the molecular function of Nostrin.

Results: Nostrin is expressed in different organs, e.g. kidney, brain, placenta or intestine. In the kidney Nostrin could be assigned to vascular endothelial cells as well as to podocytes and epithelial cells of the collecting duct and co-localizes with tight and adherens junction proteins, namely ZO-1, occludin, jam-A, VE-cadherin or claudin-5. Tightening of endothelial cell monolayer (HUVEC) with cAMP derivates leads to increased expression and membrane translocation of Nostrin whereas siRNA-mediated knockdown of Nostrin results in opening of ZO-1-positive junction strands. Co-immunoprecipitation and pulldown assays in HUVEC and Caco-2 cells revealed a strong association of Nostrin with cortactin and ZO-1.

Conclusions: We concluded that Nostrin may play a significant role in the assembly of endothelial and epithelial intercellular junctions probably by linking the cytoskeleton to the junction complex.

OSa035 SERUM GLYCOPROTEIN FETUIN-A IS DEGRAGED IN RENAL PROXIMAL TUBULAR EPITHELIAL CELLS IN RATS
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Introduction and Aims: Serum glycoprotein fetuin-A is synthesized in the liver, secreted into blood and thereby exerts potent inhibition of calcification. After the report from Dr. Ketteler et al., which clearly demonstrated the relationship between all-cause/cardiovascular mortality and low serum fetuin-A levels in hemodialysis patients, substantial attention has been focused on this molecule. Although it has been revealed that hepatic and serum fetuin-A levels are repressed by inflammatory changes or malnutrition, the degrading pathway of fetuin-A is poorly understood.

Methods: To assess the fetuin-A-degrading pathway, we performed immunohistochemical analysis using normal Wistar rat tissues. For the inhibition of proximal tubular reabsorption in 6-week old male Wistar rats, histidine-tagged soluble receptor-associated protein (His-RAP) at a dose of 20 mg/kgBW or a basic amino acid glycine served as a control in 20 mg/kgBW or 20 mmol/kgBW were administrated. A neutral aminoacid glycine served as a control in L-lysine administrated experiments. Lysosomal function was inhibited by intraperitoneal injection of leupeptin at a dose of 60 mg/kgBW.

Results: In addition to fetuin-A staining in the liver, we found dotted pattern staining of fetuin-A in renal proximal tubular epithelial cells. Because normal rat kidney dose not express mRNA for fetuin-A, this result suggested an uptake of fetuin-A in these cells. After the treatment with L-lysine, which inhibits proximal tubular reabsorption, dotted pattern staining of fetuin-A completely disappeared concomitantly with the excretion of fetuin-A into the urine. Glycine administrated control rats did not show such changes. In addition to L-lysine, we examined the effects of megalin blockade by intravenous injection of His-RAP. Similar to the results in L-lysine administrated rats, His-RAP injected rats showed a decreased staining of fetuin-A in the proximal tubular cells concomitantly with the excretion of fetuin-A into the urine. We further analyzed whether lysosomal blockade changes fetuin-A distribution in proximal tubular cells. Intraperitoneal injection of leupeptin accumulated fetuin-A in the proximal tubular cells. Immunohistochemistry revealed that fetuin-A in proximal tubular cells colocalized with LAMP-2, a lysosome marker. Western blot analysis also confirmed the lysosomal distribution of fetuin-A in the leupeptin injected rats.

Conclusions: Our results indicate that fetuin-A is absorbed by renal proximal tubular cells in a megalin dependent manner and then degraded. Kidney plays a role in fetuin-A metabolism.
RENAL SODIUM RETENTION IN CHOLESTATIC MICE IS INDEPENDENT OF ENaC IN CCD
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Introduction and Aims: The renal site of sodium retention in liver cirrhosis and the role of the amiloride sensitive sodium channel (ENaC) in the cortical collecting duct (CCD) are not clearly established as yet. We addressed this question in a new mouse model of liver cirrhosis in cholestatic mice with selective αENaC knock-out of the CCD (Scnn1a lox/lox).

Methods: ENaC knock-out (KO) and control (WT) mice underwent bile duct ligation (BDL) or sham operation (sham). Renal sodium and potassium excretion was evaluated in metabolic studies over 4 hours period.

Results: At three weeks after bile duct ligation, 30% of BDL mice displayed ascites (1-2 ml BDL(+)) whether the remaining ones did not (BDL(-)) in both groups, the WT and the selective CCD αENaC KO mice, as compared to sham mice of both groups. Urinary Na/K ratio (±SEM) was WT sham 0.9±0.2 (n=6); WT BDL(-) 0.9±0.3 (n=7); WT BDL(+) 0.1±0.1 (n=4); and KO sham 1.3±0.2 (n=6); KO BDL(-) 1.2±0.3 (n=7); KO BDL(+) 0.2±0.1 (n=3). Difference between WT BDL(+) and the other WT groups was significant (p<0.001), as the difference between KO BDL(+) and the corresponding groups (p>0.001). P<ns between WT sham and WT BDL(-), and between KO sham and KO BDL(-).

Conclusions: Ascites formation and decreased urinary Na/K ratio was observed in 30% of BDL mice independent of αENaC knock-out of the CCD (Scnn1a lox/lox). Cholestatic mice with selective αENaC knock-out of the CCD (Scnn1a lox/lox) are comparable to WT animals. ENaC in CCD is not permissive for renal sodium retention in cholestatic mice.

THE MEDULLARY SPONGE KIDNEY IS FREQUENTLY A INHERITED DISORDER: A SYSTEMATIC ANALYSIS OF A LARGE COHORT
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Introduction and Aims: Medullary sponge kidney (MSK) is a renal malformation typically associated with nephrocalcinosis and recurrent calcifications. Incomplete distal renal tubular acidosis, abnormal water reabsorption, hypocitraturia and hypercalciuria are common and concur with precalyceal cystic anomalies in triggering lithogenesis. Since very few familial cases have been reported, the disease is generally considered to be sporadic. However, observations from our previous studies on a large cohort of MSK pts have challenged the idea that the disease is sporadic and that it is always associated with a very active renal stone disease. To support these preliminary observations we investigated systematically MSK pts and their close relatives.

Methods: Until now 58 prevalent unrelated MSK patients, at random from a cohort of 112 followed-up in Verona, have been analyzed; all are calcium recurrent renal stone formers, with a i.v. urographic diagnosis of MSK. Their first and, if available, second generation relatives were investigated with renal US scan and/or i.v. urography. In probands and relatives of familial cases, plasma electrolytes, eGFR, PTH, 25(OH)VD3 and the 24-hr urine volume and excretion of protein, calcium, phosphorus, sodium, potassium, oxalate, uric acid, citrate, and morning urine pH were investigated before the introduction of any pharmacological and/or nutrimental treatment. All subjects consented to consuming a normal diet for 3 days prior to the study.

Results: We found 17 MSK probands who had at least one family member with MSK radiological pictures. Though on average 10 yrs older than probands, almost all affected relatives had an indolent disease (rare stones or colics), and some no clinical manifestation at all. As a whole in 17 families we investigated 61 subjects, of which 47 had MSK (40% males). All probands and 75% of affected relatives had bilateral MSK. Plasma phosphate and potassium were statistically significantly (ss) reduced in probands; urine pH, and 24 hr volume and calcium were all higher (ss) in probands, with a decreasing trend in MSK relatives and non-MSK relatives. While 56%, 62% and 81% of the MSK probands had hypercalciuria, hypocitraturia and UpH > 5.6, respectively, these conditions were less frequent in others with a decreasing trend in MSK- and non-MSK relatives.

Conclusions: MSK is a familial disorder in a substantial number of cases (30%), seemingly with an autosomal dominant inheritance. Most familial MSK cases have an indolent disease, probably because, though presenting the typical MSK urinary abnormalities, these are less severe than in probands.

CARDIOPROTECTIVE EFFECTS OF CALCINEURIN INHIBITION IN AN ANIMAL MODEL OF CHRONIC KIDNEY DISEASE
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Introduction and Aims: Chronic kidney disease (CKD) is associated with increased risk of death and cardiovascular events. Heart remodeling, including fibrosis, hypertrophy and decreased vascularization, is frequently present in renal diseases. Since low doses of calcineurin inhibitors (CNI) have been associated with beneficial effects on angiogenesis and cardioprotection, we aimed to investigate the impact of CNI on cardiac remodeling and function in a rat model of CKD.

Methods: CKD was induced in male Sprague Dawley rats by 5/6 resection of renal tissue. Rats were divided into five groups: sham operated rats (controls), rats with 5/6 kidney ablation (Nx) treated with CNI (cyclosporine 5.0 or 7.5, or tacrolimus 0.5 mg/kg/day) or vehicle for 14 days i.p., starting on the day of the surgery. Renal function was determined by means of creatinin clearance. Left ventricular hypertrophy and number of capillaries/regional blood volume were accessed by both morphological and echocardiographic analyses. Gene and protein expression in the heart tissue was determined by Real Time PCR and immunostaining, respectively.

Results: Renal function was significantly lower and blood pressure significantly higher in Nx rats when compared to control group. Morphological and echocardiographic analyses revealed increased left ventricular hypertrophy and decreased number of vessels/regional blood flow in Nx rats. Treatment with CNI affected neither the renal function nor the blood pressure but prevented the development of cardiac hypertrophy and improved vascularization. In addition, renal blood volume improved as confirmed by contrast agent-based echocardiography (Figure 1). Gene expression analysis evidenced an increase in pro-angiogenic (VEGF, VEGF R2) and stem cell-related (CD34, cKit-1 and SCF) genes in the hearts of CNI-treated animals, while the marker profile of inflammatory cells/inflammation was not altered after treatment.

Conclusions: In conclusion, we show for the first time that calcineurin inhibition – by using low doses of CNI – avoids CKD related heart remodeling in an animal model. Moreover, this translates independently of renal function and blood pressure into improved cardiac function. Our preclinical findings suggest that inhibition of calcineurin dependent
pathways – maybe through the development of inhibitors of calcineurin activity with cardiac specificity – might serve as a new therapeutic strategy for CKD patients at high cardiovascular risk.

**OSa039** ORAL NICORANDIL TO REDUCE CARDIAC DEATH IN HEMODIALYSIS PATIENTS WITH NORMAL CORONARY ARTERIES

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**Introduction and Aims:** Although coronary artery disease is one of the most important causes of cardiac death in patients on hemodialysis, cardiac death occurs in hemodialysis patients without significant coronary artery lesions. We investigated the protective potential of oral administration of nicorandil against cardiac death in hemodialysis patients with normal coronary arteries.

**Methods:** This study was a retrospective analysis of propensity-matched patients who had participated in a prospective cohort study. The analysis was performed in 100 propensity-matched hemodialysis patients without stenotic coronary lesions (54 men and 46 women, 64±10 years; 50 subjects who received oral administration of nicorandil (15 mg/d, nicorandil group) and 50 who did not receive oral nicorandil (control group). Absence of significant coronary lesion had been identified by coronary angiography between January 2001 and December 2004, and all participants were followed through December 2008.

**Results:** Over a mean duration of follow-up of 5.3±1.9 years, we observed 25 cardiac deaths, including 6 due to acute myocardial infarction, 11 to congestive heart failure, or 8 sudden cardiac deaths, and 15 non-cardiac deaths. The incidence of cardiac death was lower (p<0.001) in the nicorandil group (4/50, 8%) than in the control group (21/50, 42%). In multivariate Cox regression analyses revealed that increased log FGF-23 concentrations were independently associated with increased left ventricular mass index (30% increase per 1-SD increase in log FGF-23 concentration, P=0.002) and increased MPI (28.5% increase per 1-SD increase in log FGF-23 concentration, P=0.001).

**Conclusions:** Oral administration of nicorandil may improve the survival of hemodialysis patients with normal coronary arteries by preventing cardiac death.

**OSa040** SERUM FGF-23 LEVELS ARE INDEPENDENTLY ASSOCIATED WITH LEFT VENTRICULAR MASS INDEX AND INDEX OF MYOCARDIAL PERFORMANCE IN MAINTENANCE HEMODIALYSIS PATIENTS

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**Introduction and Aims:** Fibroblast growth factor 23 (FGF-23) is a phosphorus-regulating substance. Circulating FGF-23 levels increase markedly in dialysis patients and are independently associated with increased risk of mortality. Given the fact that cardiovascular disease is the leading cause of death in dialysis patients, the aim of this study was to test if elevated FGF-23 levels might be associated with left ventricular mass index (LVMI) and left ventricular index of myocardial performance (MPI) in maintenance hemodialysis patients.

**Methods:** In this cross-sectional study, plasma FGF-23 concentrations were measured using a C-terminal human enzyme-linked immunosorbent assay kit and echocardiography was performed in 128 maintenance hemodialysis patients (65 women and 63 men, mean age: 55.5±13 years, mean haemodialysis vintage: 52±10 months, all patients are on haemodialysis thrice a week).

**Results:** Mean serum FGF-23 levels were elevated when compared to age and gender matched healthy controls (954±480 vs 29±14 RU/mL, p<0.0001). Serum FGF-23 levels were higher in patients with a history of coronary artery disease (1112±433 vs 794±438 RU/mL, p=0.0001) and with aortic valve sclerosis (1221±386 vs 803±448 RU/mL, p=0.0001) than those with not. Patients with MPI (Hazard ratio, 0.123; 95% confidence interval, 0.041-0.368; p=0.0001) had higher serum FGF-23 levels than those with MPI <0.45 (1145±378 vs 657±419 RU/mL, p=0.0001). Significant correlations were recorded between serum FGF-23 levels and LVMI (r=0.285, p=0.006) and MPI (r=0.545, p=0.001). Multivariable-adjusted regression analyses revealed that increased log FGF-23 concentrations were independently associated with increased left ventricular mass index (30% increase per 1-SD increase in log FGF-23 concentration, P=0.002) and increased MPI (28.5% increase per 1-SD increase in log FGF-23 concentration, P=0.001).

**Conclusions:** Serum FGF-23 concentration is independently associated LVMI and MPI in maintenance hemodialysis patients. Further prospective studies are needed to clarify whether increased serum FGF-23 level is a marker or a potential mechanism for left ventricular involvement in patients with end stage renal disease.

**OSa041** MYOCOPHENOLIC ACID DIMINISH THE DEXAMETHASONE-INDUCED TRANSFORMATION OF VASCULAR SMOOTH MUSCLE CELLS IN OSTEOBLAST-LIKE CELLS

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**Introduction and Aims:** Arteriosclerosis is a major problem in advanced chronic kidney disease (CKD) as well as in kidney transplanted patients. Vascular calcification has long been considered to be a passive process; however recent evidence indicates actively inducing changes in vascular smooth muscle cell behavior toward an osteoblast-like phenotype. Mycophenolic acid (MPA) is a non-competitive inhibitor of inosine monophosphate dehydrogenase (IMPDH) that exerts cytostatic effects on proliferating T-lymphocytes as well as other immunomodulating effects. In this study the non-immunomodulating effects of MPA should be investigated by proving anti-arteriosclerotic properties of this substance.

**Methods:** In vitro calcification in rat vascular smooth muscle cells (VSMC) were induced with calcification medium (DMEM containing 4.5 g/L glucose supplemented with 15% FCS, 10 mmol/L sodium pyruvat, 50 μg/mL Vitamin C, and 10 mmol/L β-glycero phosphate) and dexamethasone [100 μmol/L]. Calcium deposition was monitored by Alizarin staining and quantified by O-cresolphthalein complexone method. Cbfa1 gene expression was measured by real-time PCR.

**Results:** Calcification medium (CM) with dexamethasone [100 μmol/L] induced mineralization of VSMC visualized by Alizarin staining and quantified by O-cresolphthalein complexone method. Cbfa1 gene expression was measured by real-time PCR.
Conclusions: Here, we could show that the immunosuppressant agent MPA has other, non-immunomodulating properties by diminishing transformation of VSMCs to osteoblast-like cells. Therefore, MPA might have influence on atherosclerotic alterations in the vascular wall which might have benefit for cardiovascular outcome after renal transplantation.

Introduction and Aims: The interplay of correct solute mass balances, such as that of sodium (Na+) and potassium (K+) (respectively, Na+MB and K+MB), have been investigated in new dialysis patients. However, the effects of different dialysis procedures on plasma water concentrations and their trends analyzed. Bioimpedance (BIA) measurements were determined at the start and the end of each HD session, plasma Na+ and K+ concentrations were prescribed to be the same. Plasma water Na+ and K+ trends during dialysis as well as Na+MB and K+MB were determined. At the same time, systolic (SBP) and diastolic blood pressure, mean arterial pressure (MAP) and heart rate trends during dialysis were analyzed. Plasma volume (PV) changes were computed from plasma volume, mean arterial pressure (MAP) and heart rate trends during dialysis.

Methods: The present study has a crossover design: 11 stable anuric HD patients underwent 2 bicarbonate HD sessions (~4h and ~8h) in a random sequence, always at the same interdialytic interval, at least one week apart. The volume of blood and dialysate processed, V UF and dialysate Na+ and K+ concentrations were prescribed to be the same. Plasma water Na+ and K+ trends during dialysis as well as Na+MBs and K+MBs were determined. At the same time, systolic (SBP) and diastolic blood pressure, mean arterial pressure (MAP) and heart rate trends during dialysis were analyzed. Plasma volume (PV) changes were computed from plasma total protein concentrations and their trends analyzed. Bioimpedance (BIA) measurements were determined at the start and the end of each HD session, injecting 800 µA at 50 kHz alternating sinusoidal current with a standard tetrapolar technique. The BIA variables measured were resistance (R) and reactance.

Results: Dialysis sessions were uneventful; a better haemodynamic stability, mainly due to statistically significantly lower intradialytic decreases of SBP (∼−4 ± 2 vs −20 ± 15 mmHg, P < 0.02) and MAP (∼−1 ± 1 vs −8 ± 6, 11 mmHg, P < 0.04), was achieved in the 8h sessions with respect to the 4h sessions, in spite of not different mean V UF (2.9 ± 0.9 vs 2.9 ± 0.8 l; P=NS) and mean Na+MBs (∼−38 ± 17 vs −38 ± 137.0 mmol; P=NS). PV reductions were statistically significantly lower in the 8h sessions. The latter were associated with post-dialysis R values, ΔR values, and percent increase of R values statistically significantly higher than the corresponding values of the 4h dialysis sessions.

Conclusions: The present highly controlled experiments using a crossover design and precise Na+MB and K+MB controls showed that a better haemodynamic stability was achieved in the 8h sessions with respect to the 4h ones, in spite of not different mean V UF and Na+MBs. Thus, other pathophysiological mechanisms, such as better PV preservation, must be investigated in order to explain the better haemodynamic stability peculiar to long-hour slow-flow HD treatments. Finally, if higher R values may represent a proxy of a correct dry body weight, it remains a matter of future research.
levels during standard HD sessions with low-molecular-weight-heparin – enoxaparin (ENX) used as an anticoagulant in comparison to those during HD without systemic heparinization.

Methods: Nineteen clinically stable maintenance HD pts (9 men; aged 58 ± 18.8 yrs; dialyzed 3 x wk for at least 4 mo) were enrolled and served as a case and a control group. First HD sessions were performed as usual – with no-reuse low-flux polysulfone membrane and an iv bolus of 40 (20-60) mg of ENX given on HD initiation. Consecutive HD sessions were performed in the same pts with the use of a new anti-thrombogenic heparin-grafted HeprAN hydrogel membrane derived from polyacrylonitrile AN69 ST (Evodial, Gambro Diaverum) and no ENX administration. Blood samples were collected from a v-fistula circuit before the ENX-anticoagulated or “Evodial” HD (T0) and, then, after 10 min (T10) and 120 min (T20) of HD procedures from the circuit. Plasma MPO levels were measured using a referential ELISA kit from R&D Systems.

Results: During standard ENX-anticoagulated HD plasma MPO levels markedly increased in all 19 pts (7.6-fold at T10 vs baseline, 5.1-fold at T20 vs baseline) and were a median (min-max): T0 72.5 (2.53-298) ng/ml, T10 531 (144-1776) ng/ml, T20 367 (99.5-1023) ng/ml, ANOVA P < 0.0001. The percentage increments in plasma MPO did not correlate significantly with the ENX dose per kg b.w. During HD with the Evodial dialyzer MPO levels remained statistically unchanged: To 71.4 (20.8-374) ng/ml, T10 138 (31.0-646) ng/ml, T20 105 (34.7-570) ng/ml, ANOVA P > 0.5. There were no changes in WBC count during both HD sessions (data not shown).

Conclusions: 1. Enoxaparin administered for HD anticoagulation induces a marked and prolonged increase in plasma MPO as a likely result of the release of the cytotoxic enzyme from its endothelial stores; the effect may be important and vasoconstrictive. 2. Overdialytic plasma MPO levels should not be regarded as a measure of dialyzer membrane biocompatibility during heparin-anticoagulated HD.

**OSa046 USEFULNESS OF HCV-RNA DETECTION IN ULTRACENTRIFUGED SERUM FOR THE DIAGNOSIS OF OCCULT HCV INFECTION IN HEMODIALYSIS PATIENTS**

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Introduction and Aims: Detection of HCV-RNA in PBMC identifies occult HCV infection in 45% of anti-HCV and serum HCV-RNA negative hemodialysis patients with abnormal liver enzymes (J Am Soc Nephrol 2008; 19:2288). Patients with occult HCV may have very low levels of circulating viral particles only detectable after serum ultracentrifugation (J Virol 2007 81:7710). The aim of the study was to compare the usefulness of testing for HCV-RNA in PBMC and in serum (after ultracentrifugation) for diagnosing occult HCV infection in repeatedly anti-HCV and serum HCV-RNA negative hemodialysis patients with abnormal liver enzymes.

Methods: HCV-RNA was tested in PBMC and in 2 ml of ultracentrifuged serum by real-time PCR (J Virol 2007 81:7710). A total of 82 hemodialysis patients who fulfilled the inclusion criteria were included in the study. Mean age 63,38 ± 15 years, 58 (70,7%) were males, 30% of them were Diabetic, mean HD time 42,01±43,0 months.

Results: HCV-RNA in PBMC was detected in 30/82 (36,5%) of the patients, indicating an occult HCV infection. The median viral load in PBMC was 3.9 x 10^5 copies of HCV-RNA/jg total RNA. After serum ultracentrifugation it was found that 15/82 (18,3%) hemodialysis patients were HCV-RNA positive with a median load of 110 copies of HCV-RNA/ml serum (range: 25-1160 copies). Combining the results of both techniques, 40/82 (48,8%) HD patients had an occult HCV infection. Of these patients, 7 were simultaneous positive for viral RNA in PBMC and in ultracentrifuged serum, 23 were only positive for viral RNA in PBMC and the remaining 8 patients had only serum HCV-RNA (after ultracentrifugation). Thus, detection of HCV-RNA in ultracentrifuged serum samples allowed diagnosis of occult HCV infection in 843 (19%) HD patients with undetectable HCV-RNA in PBMC. Liver enzymes: x ALT 35.91±2.94, in Occult HCV+ vs 25.90±1.19 in HCV negative. Age, etiology of renal disease, HD time or antecedents of blood transfusion did not differ between patients with or without occult HCV infection.

Conclusions: Testing for HCV-RNA in serum after ultracentrifugation identifies up to 19% of hemodialysis patients with occult HCV infection who tested negative for viral RNA in PBMC. Presence of HCV-RNA in PBMC in the absence of serum HCV-RNA (after ultracentrifugation) may be explained by the decrease in HCV-RNA load observed with the hemodialysis.
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**OSa047** REDUCTION OF MMSE SCORE OVER TIME IS MORE SEVERE IN CHRONIC HEMODIALYSIS PATIENTS THAN IN ELDERLY PATIENTS

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**Introduction and Aims:** Cognitive impairment is common in chronic hemodialysis patients (HDP) but the course of cognitive function deterioration over time is unknown. In this observational, longitudinal study, we compared the eventual changes of MMSE score over time in chronic HDP with that elderly patients (EP) and defined the variables associated with such changes.

**Methods:** HDP of the Hemodialysis Service and EP of the Geriatrics Day Hospital of the Catholic University of Rome underwent assessment of cognitive function through the Mini Mental Status Examination (MMSE) at baseline and after one year. For EP, eligibility requirement was age ≥ 65 years. For both groups exclusion criteria were dementia based on DSM-IV criteria, alcohol or substance abuse, decompensation of physical conditions requiring hospital admission. In HDP, the MMSE was administered during a midweek hemodialysis session and in EP in the morning. A MMSE score ≤ 23 was indicative of cognitive impairment. Patients were stratified at baseline and after one year in 3 groups according to MMSE score: normal cognitive function [normal]: ≥ 24; mild cognitive dysfunction [mild]: 18-23; moderate-severe cognitive dysfunction [moderate-severe]: < 18. Categorical variables were compared using the χ2 test. Univariate tests and multivariate linear regression analysis were performed.

**Results:** One hundred twenty-eight HDP and 194 EP were screened for study participation. Of these, 28 and 24 were not included because of one year follow- up. HDP were younger than elderly patients, were more frequently affected by hypertension, diabetes, COPD, heart failure and had lower hemoglobin levels. At one year, the median reduction of MMSE score was more severe in HDP than EP patients switched from normal (MMSE > 23) to mild (MMSE 18-23) or severe (MMSE < 18) MMSE group.

At multivariate analysis, baseline MMSE was negatively correlated with hypertension (p=0.015), angina (p=0.009) and Beck Depression Inventory (p=0.046) and positively correlated with education (p=0.019) and male gender (p=0.019). At the multivariate analysis we found that peripheral vascular disease (p=1.626; p=0.004) was associated with deterioration of the cognitive function over time.

**Conclusions:** The MMSE score reduction at one year is more severe in HDP than in EP. The causes of such event remain unknown. The results of this study suggest the importance to strictly monitor the cognitive function in HDP.

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**CKD-related bone disease**

**OSa048** CORONARY CALCIFICATION AND BONE HISTOMORPHOMETRIC ANALYSIS PRIOR AND 1-YEAR AFTER PARATHYROIDECTOMY IN DIALYSIS PATIENTS

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**Introduction and Aims:** Alterations of bone and mineral metabolism in dialysis patients may contribute to the development of vascular calcification, a prognostic marker of mortality in that population. The aim of this study is to evaluate the bone remodeling disorders after parathyroidectomy (PTX) for the treatment of severe secondary hyperparathyroidism (sHPT) and its association with the progression of coronary artery calcification (CAC) in dialysis patients.

**Methods:** Twenty-five dialysis patients with sHPT entered a 1-year prospective study that included bone biopsy, multi-slice coronary tomography, bone densitometry and laboratorial analysis prior and 1 year after PTX. Nineteen patients (7 M/12 F; age 45.05±11.2; time on hemodialysis 105.37±46.7 mo) completed the study. Undecalcified bone biopsy was previously labeled by tetracycline and followed by histomorphometric analysis. CAC was defined as calcium score (CaS) > 30 AU. Change of CaS > 15% was defined as CAC progression. Cumulative calcium salts and calcitriol doses were calculated during the study.

**Results:** Baseline and 1 year data can be seen in the table. In the study group, 6 patients (31%) did not have CAC neither in baseline nor 1 year after PTX. Thirteen patients (69%) had CAC and 6 of them (46.2%) did not present progression. Seventeen patients completed the study with bone biopsy prior and one year after PTX. At baseline, all patients showed hyperparathyroidism bone disease, 75.6% of whom switched to adynamic bone disease 1 year after PTX. There was no association between changes in CAC progression and bone disease pattern. Cumulative calcium salts and calcitriol doses were 425.8±228.7g and 380.5±292.6ug, respectively, and no correlation between either doses and the delta of CaS was observed.

**Baseline and one year follow up data**

|                      | Baseline | One year | p    |
|----------------------|----------|----------|------|
| iCalcium (mmol/L)    | 1.33±0.1 | 1.19±0.1 | <0.001|
| Phosphorus (mg/dL)   | 6.8±2.1  | 5.3±1.6  | 0.006 |
| Alk. Phase (U/L)     | 781±66   | 109±42   | <0.001|
| iPTH (pg/mL)         | 1987±551 | 752±77   | <0.001|
| CRP (mg/dL)          | 0.82±1.0 | 1.33±2.26| NS    |
| Total Col (mg/dL)    | 142±44   | 184±38   | 0.002 |
| OPG (pg/mL)          | 420±227.3| 330±242.6| 0.024 |
| Rank-L (pg/mL)       | 39.27±40.11| 50±1±51 | <0.001|
| CaS (AU)             | 723±1122 | 789±177 | 0.034 |
| BMD L2L4 (g/cm2)     | 1.02±0.279| 1.17±0.269| <0.001|
| BMD Neck (g/cm2)     | 0.82±0.211| 0.97±0.234| <0.001|
| BV/TV (%)            | 14.9±9.6 | 17.75±11.6| NS    |
| BFR/BS (μm²/μm²/day) | 0.16±0.09 | 0.22±0.03 | 0.001 |

OPG = Osteoprotegerin, Rank-L = Soluble receptor activator of nuclear factor-κB, BMD = bone mineral density, BV/TV = bone volume/tissue volume, BFR/BS = bone formation rate/bone surface

**Conclusions:** PTX in dialysis patients with severe sHPT leads to a high prevalence of adynamic bone disease, which cannot be attributed to the CAC progression, during the first year after the surgery.

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**The CALCIMIMETICS AM641 PREVENTS CORTICAL BONE LOSS AND POROSITY IN 5/6-NEPHRECTOMIZED RATS ON HIGH PHOSPHATE DIET**

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**Introduction and Aims:** Chronic kidney disease (CKD) is characterized by increased parathyroid hormone (PTH) levels which impacts cortical bone sites leading to high fracture rates. Calcimimetics reduce PTH secretion via actions on the parathyroid gland calcium-sensing receptor. We hypothesized...
that calcimimetics would prevent the cortical porosity and associated bone effects in hyperparathyroid CKD rats. **Methods:** Male, 5/6 nephrectomized rats (5/6Nx) were fed a high phosphate (1.2% Pi) diet and treated daily (PO) with the calcimimetic AMG641 (3, 1, 0.3 mg/kg) or vehicle; n = 9, 6, 7, and 7, respectively; sham-operated rats served as controls; n = 5. Treatment and 1.2% Pi diet were initiated 3-weeks post 5/6Nx. Micro computed tomography (μCT), cortical histomorphometry, serum calcium (Ca) and PTH were analyzed after 5-weeks of treatment while on the 1.2% Pi diet. **Results:** Serum PTH levels were elevated in vehicle-treated CKD rats compared to sham controls, 399±48 pg/mL vs. 416±39 pg/mL, respectively (p<0.05). AMG641 treatment dose-dependently reduced the CKD-induced increase in serum PTH levels and maintained normal levels at the 3 mg/kg dose; 376±351 pg/mL at 0.3 mg/kg (n.s. vs. CKD, p<0.05 vs. sham), 2047±455 pg/mL at 1 mg/kg (n.s. vs. CKD, n.s. vs. sham), and 573±212 pg/mL at 3 mg/kg (p<0.05 vs. CKD, n.s. vs. sham). Consistent with elevated serum PTH, marked cortical bone porosity was noted in the mid-shaft femur of CKD rats, compared to sham controls; 9.1±4.4% vs. 0.32±0.23%, respectively (p<0.05). AMG641 prevented mid-shaft femur cortical porosity in CKD rats; 4.2±2.2% at 0.3 mg/kg (n.s. vs. CKD, p<0.05 vs. sham), 0.9±0.5% at 1 mg/kg (n.s. vs. CKD, n.s. vs. sham), and 1.9±0.5% at 3 mg/kg (n.s. vs. CKD, n.s. vs. sham). Vehicle CKD rats had significantly reduced cortical BMD compared to sham controls; 929±34 mg/cm² vs. 1030±4 mg/cm², respectively (p<0.05). AMG641 treatment normalized cortical BMD, compared to sham controls at the 1 and 3 mg/kg doses; 963±12 mg/cm² at 0.3 mg/kg (n.s. vs. CKD, p<0.05 vs. sham), 992±29 mg/cm² at 1 mg/kg (n.s. vs. CKD, n.s. vs. sham), at 3 mg/kg 1002±3 mg/cm² (n.s. vs. CKD, n.s. vs. sham). Serum Ca was decreased by AMG641 treatment at the lower doses of AMG641 did not affect serum Ca levels; 9.4±0.2 mg/dL at 0.3 mg/kg (n.s. vs. CKD, n.s. vs. sham). Importantly, beneficial effects of AMG641 on cortical bone were evident at doses that did not lower serum Ca.

Conclusions: These data show that AMG641 prevents the cortical porosity induced by secondary hyperparathyroidism associated with uremia in CKD rats, and suggest that calcimimetics may be useful in the clinical management of CKD-MBD.

Disclosure: Employee and stock holder Amgen Inc.
This association remained significant even after adjustment for traditional cardiovascular risk factors and CKD stage.

Indeed, patients in the highest serum phosphorous quartile (>4.3 mg/dl) experienced a 117% increase in the risk of the occurrence of the composite endpoint when compared to patients with serum levels of phosphorous of 3.3-3.8 mg/dl (Hazard Ratio 2.17; 95%; Confidence Interval 1.62-2.93; p<0.001).

Conclusions: These analyses lend support to the hypothesis that serum phosphorous levels at baseline might accelerate residual renal function deterioration and increase the risk of death in patients with mild to moderate renal function impairment.

THE LINK BETWEEN MIA AND INCIDENT HIP FRACTURE IN HEMODIALYSIS PATIENTS

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Introduction and Aims: It is well established that malnutrition, inflammation, and atherosclerosis (MIA) syndrome is a major cause of morbidity and mortality in end-stage renal disease, which is often observed in hemodialysis (HD) patients. It is likely that elevated levels of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, and TNF-α play a central role in the vicious cycle of MIA. Given that all these cytokines are bone resorbing cytokines, there might be a link between MIA and bone fragility. The aim of this study is to know if MIA is a risk factor of incident hip fracture in Japanese HD population.

Methods: In this observational study, we used the nationwide dialysis registry of Japanese Society for Dialysis Therapy (Japanese Renal Data Registry) performed at the end of year 2007 and 2008. The prevalence of hip fracture was studied in these consecutive surveys, making it possible to calculate the main outcome, the incidence of hip fracture by subtraction in those who had never experienced hip fracture at the time of 2007 (JRDR-08102). Enrolled patients are those who underwent HD 3 times/week and those who had never experienced hip fracture at the time of 2007 (JRDR-08102) and followed up until 2008. Those who had a history of peritoneal dialysis were excluded from the analysis.

Results: The number of enrolled patients was 89,793. Lower serum albumin and higher CRP were significantly associated with higher odds of having incident hip fracture. Prior history of CVD was associated with higher odds of fracture (odds ratio 95%, CI: 1.17-1.54). There was a monotonous positive relationship between the numbers of the components of MIA syndrome and the adjusted odds ratio for fracture.

Conclusions: In this largest study in the world, we found a close link between MIA and incident hip fracture. This link would give rise to a new concept, MIA-bone syndrome. This novel concept might help us to understand why the history of fracture is associated with high mortality in HD patients.

THE CLINICAL CHARACTERISTICS OF CFHR5 NEPHROPATHY IN 104 INDIVIDUALS FROM 16 CYPRIOT KINDREDS. AN OLD BUT NEWLY RECOGNIZED DISEASE THAT CAN CLINICALLY MIMIC IGA, BERGER’S NEPHROPATHY

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Introduction and Aims: Following the recent work by Drs DP Gale and colleagues in London, on the genetic aspects of “CFHR5 Nephropathy” on several Greek-Cypriot patients in London and Cyprus, we have been able to expand quickly on this new renal disease using the DNA bio bank of inherited renal diseases that has been built in Cyprus over the last 18 years. The causal mutation is a duplication of exons 2-3 of complement factor H related protein 5 (CFHR5) gene that results in reduced affinity for glomerular-bound complement.

Methods: We have screened genetically 212 members from 16 affected Cypriot families, using a simple molecular (PCR) test. All the “new found” mutation carriers were clinically examined. Seventeen renal biopsies are available for study.

Results: Of the 162 patients at risk, 104 (64.2%) carry this mutation (MC). 10 patients (9.6%), at various ages (12-88 yo) show no urinary abnormalities. The great majority, 61 patients (58.7%), only show microscopic hematuria, while 33 patients have developed additional proteinuria (32.7%). Of these 33 proteinuric patients 29 have gone on to develop renal failure and 18 have reached ESRD. Of these 18 ESRD patients, 15 are males (83%) and 3 are females (17%). The characteristic biopsy finding is mesangial increase with only C3 deposits in the mesangium and sub-endothelial area and no immunoglobulins. Glomerular basement membranes are of normal thickness except in one family where affected members also show thin basement membrane nephropathy of unknown aetiology and no heterozygous COLA3/COLA4 mutation. Serum levels of complement C3 and C4 were normal in all affected individuals studied.

It is of interest that many of these patients have exhibited in childhood and adolescence several episodes of syphymyngitic macroscopic hematuria that were then followed by persistent microscopic hematuria. The clinical picture at the time was very similar to IgA nephropathy and in the absence of a renal biopsy several medical reports were written suggesting that Berger’s disease may have been the underlying explanation.

Conclusions: CFHR5 nephropathy is an old but only recently explained inherited renal disease that should widen our approach to childhood and adolescence episodic macroscopic hematuria and microscopic hematuria.
from IgA nephritis, post infectious GN in general, angitis and mutations in the COL4A4/COL4A3 and COL4A5 genes to also include CFH5R, an alternate pathway mediated nephritis.

**OSa054 LOW CYCLOPHOSPHAMIDE (CYC) DOSE AND PLASMA EXCHANGE (PLEX) A LESS TOXIC INDUCTION TREATMENT OF ANCA ASSOCIATED VASCULITIS (AAV)**

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**Introduction and Aims:** The AAV treatment related toxicity (25-50%) is still a serious outcome influencing factor mostly due to over-immunosuppression. Despite the growing evidence of the involvement of ANCA in the pathogenesis of vasculitis, the use of plasma exchange (PE) in AAV is not commonly accepted in patients (pts.) with plasma creatinine below 500 μmol/L. We combined low dose of CYC and PLEX in order to minimize the rate of severe infections/sepsis, which is an important patient survival factor in the first 4 months of AAV.

**Methods:** A prospective cohort study of all AAV pts., referred to our centre, was conducted between 2000 and 2009. All pts. had AAV on the basis of a positive ANCA titre and a compatible clinical syndrome. PE use was determined on the basis of active lesions on renal biopsy and ANCA titres. Standard therapy for all pts. consisted of prednisolone + mycophenolate mofetil for at least 2yrs. Follow up of the cytokine assay. All patients received induction treatment with prednisolone + mofetil was given for maintenance of remission after 4 months.

**Results:** 121 pts. were followed for a mean of 3 years (range 0.5-8.5; 368 patient years). 60% were male, 81% were PR3-ANCA and 19% were MPO-ANCA positive. 47% had an initial creatinine ≥ 500 and 53% < 500 μmol/L. 36% were aged ≥ 65 years and 65% < 65 years. 97 pts. with impaired renal function received plasma exchange (mean 7 (range 5-11)). Only 3 pts (2.4%) died during CYC treatment – 1 sepsis, 1 AMI, 1 lung hemorrhage. Totally 9 pts. (7.4%) died initially within 12 months of active vasculitis (5 pts. from the high creatinine group) and six more pts. died within 7 years without signs of active vasculitis. (Total mortality of 12.4%). Eleven (9%) developed end stage renal failure. 36 relapses occurred during the 9 years of follow up, 12 during the first year after induction. Pts. aged < 65, < 500 had significantly better dialysis free survival.

**Conclusions:** Thus, the use of induction therapy with low dose CYC and PLEX resulted in a high remission rate with a good preservation of renal function and low rate of complications providing evidence of possible sparing effect of PLEX in induction therapy in AAV with renal disease.

**OSa055 DIFFERENT CYTOKINES PRODUCED WITHIN THE KIDNEY HAVE DIFFERENT INFLUENCE IN THE OUTCOME OF PAUCI IMMUNE RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS (RPGN)**

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**Introduction and Aims:** Multiple cytokines are produced within the kidney during the pathogenesis of pauci immune RPGN, playing different roles during the course of the disease, either by introducing inflammation and fibrosis, or by alleviating kidney damage. Detection of the panel of cytokines may give valuable information about disease pathogenesis and prognosis.

**Methods:** First morning urine samples from 38 patients with pauci immune RPGN, collected at day of renal biopsy. [17 female, age 59.5yrs (25-80)], and 10 healthy controls, were used to detect EGF, TGFβ1 and VEGF by hs-ELisa, and IL-1β, IL-8, IL-17, IL-15, MCP-1, MIP-1α, MIP-1β by a multiplex cytokine assay. All patients received induction treatment with prednisolone+mofetil+cytophosphamide for 3 months, followed by maintenance treatment with prednisolone+mofetil for at least 2yrs. Follow up of the patients was 39.4±4.5months.

**Results:** Urinary levels of EGF had a significant negative correlation with the percentage of global sclerosed glomeruli and fibrinous crescents (r=0.5, p<0.01 and r=-0.7, p<0.001, respectively), urinary concentration of VEGF also correlated negatively with the percentage of global sclerosed glomeruli (r=-0.6, p=0.003), and TGF-beta urinary levels had a very strong correlation with the degree if interstitial fibrosis (r=0.9, p<0.0001).

Cytokines whose increased urinary levels were associated with response to treatment and improvement of renal function were EGF, IL-4, IL-8 and IL-9. Increased urinary levels of IL-6, IL-15, VEGF, TGF-β, MIP-1β were associated with poor outcome of renal function. In multiple regression analysis, the response to induction therapy (first 3 months) was correlated with the urinary excretion of IL-6 and VEGF, (r2=0.3, p<0.001 and r2=0.6, p<0.001).response to maintenance treatment (end of follow up) correlated only with the IL-6 urinary levels (r2=0.2, p=0.03).

**Conclusions:** In the presence of pauci immune RPGN multiple cytokines are produced in the kidneys and excreted in the urine. They play significant role in the development of histological damage and influence in several ways disease outcome, while IL-6 and VEGF seem to be the main predictors of response to treatment.

**OSa056 DECLINE IN RENAL FUNCTION IS A ‘HIDDEN DOMAIN’ OF DISEASE ACTIVITY IN NONRenal SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)**

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**Introduction and Aims:** Renal involvement is common but not universal in SLE, the prevalence of renal disease varies from 31% to 65%. Clinical manifestation of lupus nephritis (LN) are well-known features of disease activity with urinary abnormalities being most common. Most patients with SLE were found to have histologic evidence of LN on renal biopsy, even in the absence of urine or serum abnormalities. This has been termed silent lupus nephropathy and is typically nonprogressive. Most validated SLE disease activity indices do not take GFR as a variable of activity in absence of LN.

We aimed to investigate GFR in SLE patients without LN and to determine the predictors of GFR in such patients.

**Methods:** We evaluated 77 SLE patients without LN – age (M (SD)) 34.35 (14.15), 63 (81.8%) female, disease duration 6.93 (6.88) years. Disease activity was measured by SLEDAI and physician global assessment (PGA), cumulative activity by adjusted mean SLEDAI, damage by SLICO/ACR index (DI). GFR was estimated by CKD-EPI equation, routine clinical and laboratory parameters collected. Data was compared with 52 patients with SLE and LN. Statistical analysis included ANOVA, Spearman correlation and stepwise linear regression model.

**Results:** SLE activity was mild in 21 (27.2%), moderate in 28 (36.4%) and severe in 28 (36.4%) patients. SLEDAI score (M (SD)) was 7.14 (5.77), DI score 1.39 (1.51). 30 (39%) were free of damage. Corticosteroids were prescribed for 66 (85.7%), cytotoxics for 10 (13%), NSAIDs for 46 (59.7%) patients. Disease activity was significantly lower than in patients with LN (SLEDAI 11.15 (6.89), p<0.001); while damage was not. GFR was estimated as 90.59 (22.73) [range 45.3 to 133.9] ml/min/1.73m², significantly higher than in patients with LN (59.72 (29.82), p<0.001; 6 (7.8%) patients had GFR <60 ml/min/1.73m². GFR correlated negatively with SLEDAI (r=-0.466, p<0.001), PGA (r=-0.307, p=0.009), cumulative SLE activity (r=0.356, p=0.012), DI (r=0.522, p=0.007), blood pressure (r=0.406, p<0.001), antinuclear antibodies titer (r=0.241, p=0.043), patient’s age (r=0.649, p<0.001) and age of SLE onset (r=0.575, p<0.001). When included in regression model, only age and SLEDAI score remained significant predictors for GFR with respective B (95% CI) -0.951 (-1.248 to -0.655) and -0.884 (-1.584 to -0.184) and model summary adjusted R-square 0.488. No influence of SLE treatment modalities on GFR was detected. In patients with LN no correlation of GFR was seen for SLE activity, damage or age.

Such differences indicate that in SLE patients without LN GFR is dependent on age, GFR reflects renal disease progression independent of SLE activity. GFR in SLE reflects extent and severity of glomerular damage.

**Conclusions:** GFR in nonrenal SLE depends on disease activity, thus reflecting a ‘silent domain’ of renal involvement. Precise mechanisms of nonrenal active SLE influence on GFR decline remains to be disclosed.
REDUCED CYCLOPHOSPHAMIDE REGIMENS FOR ANCA-ASSOCIATED VASCULITIS ARE ASSOCIATED WITH AN INCREASED RISK OF RELAPSE BUT NOT DEATH: AN INDIVIDUAL PATIENT META-ANALYSIS OF THREE TRIALS

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Introduction and Aims: New treatment regimens in ANCA associated vasculitis (AAV) have concentrated on reducing toxicity associated with cyclophosphamide (CYC) by reducing cumulative CYC exposure. Individual trials suggest non-inferiority but are limited by low event rates. We conducted an individual patient data meta-analysis of 3 randomised controlled trials with updated long-term follow-up data that compared a standard, oral CYC regimen to a reduced CYC exposure regimen.

Methods: We examined the time to first relapse in patients with AA V regimen to a reduced CYC exposure regimen. We assessed the association between a reduced exposure and relapse with a proportional hazards model adjusted for age, sex, diagnostic subgroup, initial disease severity, baseline serum creatinine, involvement of the upper respiratory system, lower respiratory system, and nervous system and the effects of between trial variability. We used an identical model to assess CYC regimen on mortality.

Results: Three hundred ninety-eight patients were available for analysis. Seventy of 197 (35.5%) patients in standard CYC regimens and 105/201 (52.2%) in reduced exposure regimens had a relapse of AAV. Death occurred in 32/197 (16.2%) of patients in the standard regimens compared to 31/201 (52.2%) in reduced exposure regimens. We compared methotrexate to oral CYC, one a shortened course of oral CYC, and one an IV pulse regimen to oral CYC. We assessed the association between a reduced exposure and relapse with a proportional hazards model adjusted for age, sex, diagnostic subgroup, initial disease severity, baseline serum creatinine, involvement of the upper respiratory system, lower respiratory system, and nervous system and the effects of between trial variability. We used an identical model to assess CYC regimen on mortality.

Conclusions: Reduced exposure regimens, were not associated with an increased risk of death (HR 1.10; 95% CI 0.92 – 1.31; p=0.30).

The addition of Saquinavir to induce and maintain remission allowed a great reduction in steroids, TAC and other IS drugs (25 to 100%), while a steroid-resistant case remained relapse-free for 12 months. No side effects were observed apart from mild diarrhea. The results obtained were relevant considered the severity of the cases enrolled.

Experimental pathology and renal histopathology

BACTERIAL CPG-DNA ACCELERATES ALPORT GLOMERULOSCLEROSIS BY INDUCING A M1 MACROPHAGE PHENOTYPE AND TNF-MEDIATED PODOCYTE LOSS

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Introduction and Aims: Loss of function mutations in the α or α chain of type IV collagen (COL4A) cause autosomal recessive Alport nephropathy in humans and mice which is characterized by progressive glomerulosclerosis. The mechanisms that determine disease progression remain unclear. We found that the progression of kidney disease in Col4a3-deficient mice is associated with a robust influx of immune cell subsets including non-activated macrophages. We hypothesized that transient immune recognition of bacterial products would accelerate the progression of Alport nephropathy by enhancing intrarenal inflammation.

Methods: At 4 weeks of age Col4a3-deficient mice on a 129SvEv genetic background were received intraperitoneal injections of normal saline (vehicle), LPS (10 μg) or CpG-ODN (40 μg) every alternate day for 2 weeks. An additional cohort of CpG-DNA-treated Col4a3-deficient mice was intraperitoneally injected with 100 μg etanercept every other day from week 4 to 6 to block TNF-α. Lifespan was monitored and renal pathologic evaluations were performed.

Results: A mean survival time was 76 days in saline- and LPS-treated Col4a3-deficient mice and disease progression was not affected, whereas an equitent dose of CpG-DNA reduced the overall life span (46 days, p=0.006) of Col4a3-deficient mice and accelerated glomerular sclerosis and loss of WT-1 positive podocytes (Histopathologic and electron microscopy analysis). This effect of CpG-DNA was associated with a significant increase of intrarenal F4/80+CD11c+ macrophages (p<0.001 versus saline-treated mice), especially CD11b+/Ly6C0 macrophages (p<0.05 versus saline-treated mice). Immunohistochemical and RT-qPCR data revealed high expression of iNOS, TNF-α, IL-12, and CXCL10 in kidney of CpG-treated Col4a3-deficient mice. The induction of TNF-α was essential for CpG-DNA-induced acceleration of Alport nephropathy because concomitant TNF-α blockade with etanercept entirely abrogated the CpG-DNA effect (Lower glomerular sclerosis scores and increased number of WT-1 positive podocytes).
Conclusions: We concluded that non-activated macrophages accumulate during the progression of Alport nephropathy. Systemic exposure to CpG-DNA modulates the phenotype of intrarenal macrophages towards classically-activated (M1) macrophages which enhance intrarenal inflammation and the progression of Alport nephropathy, e.g. via the secretion of TNF. Thus, factors that modulate the phenotype of renal macrophages can affect the progression of Alport nephropathy, and potentially of other types of chronic kidney diseases.

**OSa060 REGULATION OF THE PRO-APOTOTIC BAD PROTEIN BY COX-2 DERIVED PGE2 IN THE RENAL MEDULLA**

Christoph Küper, Daniela Steiner, Franz-Xaver Beck, Wolfgang Neugebauer

Introduction and Aims: The results indicate that, during osmotic stress, COX-2 de-

bad by siRNA lowered apoptotic indices during hypertonicity and blunted

PKA, which mediates phosphorylation of BAD at Ser155. Knockdown of

culture experiments, a rise in medium tonicity to 700 mosmol/kg H2O

activity during water-deprivation was increased in COX-2-/- mice. In cell

particularly in the inner medulla. Accordingly, inner medullary caspase-3

expression and phosphorylation of BAD was analyzed by immunoblotting

transduction pathways in vitro.

Methods: In water-deprived COX-2+/+ mice, PGE2 synthesis was signifi-

cantly increased compared to COX-2-/- mice with free water access. This

was accomplished by strongly increased phosphorylation of BAD. Compared

to COX-2+/+ animals, PGE2 synthesis as well as phosphorylation of BAD

was significantly decreased in COX-2/- mice, both during water-deprivation

or free water access, while overall expression of BAD was slightly higher,

particularly in the inner medulla. Accordingly, inner medullary caspase-3

activity during water-deprivation was increased in COX-2/- mice. In cell

culture experiments, a rise in medium tonicity to 700 mosmol/kg H2O

strongly induced apoptosis, which could be partially reverted by addition of

PGE2 to the medium. This effect was caused by PGE2-induced activation

of PKA, which mediates phosphorylation of BAD at Ser155. Knockdown of

BAD by siRNA lowered apoptotic indices during hypertonicity and blunted

the protective effect of PGE2.

Conclusions: The results indicate that, during osmotic stress, COX-2 de-

vived PGE2 induces PKA mediated phosphorylation, and hence inactivation,

of pro-apoptotic BAD protein. This mechanism favours cell survival par-

ularly during diuresis in the renal inner medulla. These findings may

provide novel insights into the mechanism of NSAID-induced renal

medullary injury.

**OSa061 MEMBRANOUS NEPHROPATHY (MN) INDUCED BY ARYLSULFATASE B ENZYME REPLACEMENT THERAPY (ERT)**

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Introduction and Aims: The type-M phospholipase A2 receptor (PLA2R)

has recently been identified as a candidate antigen in 60-70% of idiopathic

MN. The nature of antigens involved in secondary MN remains elusive.

This work identifies a new allo-antigen responsible for secondary MN.

Methods: We have investigated a patient with mucopolysaccharidosis

type VI due to complete deficiency of aryl sulfatase B (ASB). Enzyme

replacement therapy (ERT) started in January 2007 at the age of 4

years (dose 1mg/kg/week) dramatically improved clinical manifestations

of disease and simultaneously reduced urinary glycosaminoglycans. One

week after orthopedic surgery (June 2008) the patient developed nephrotic

syndrome (proteinuria 38g/l; albuminemia 11g/l) without renal insufficiency.

The renal biopsy revealed subepithelial glomerular deposits of IgG and C3

electron dense deposits characteristic of MN. Antibodies against rhabas

were searched for by ELISA and IgG subclass identification was performed

by Western blotting in the serum. Specific rabbit and sheep anti-rhabas

antibodies were used to detect rhabas in the glomerular deposits.

Results: High titters of circulating anti-rhabas antibodies were measured

by ELISA. IgG4 subclass was the most prominent by Western blotting while

all IgG subclasses were detected in the biopsy sample. Immunofluorescence

showed subepithelial granular deposits of rhabas along capillary loops.

No staining of rhabas was seen in biopsy specimens from patients with

idiopathic MN or other glomerular diseases. The specificity of rhabas

staining was shown by complete inhibition of staining after preincubation

of anti-rhabas antibody with an increasing concentration of rhabas protein.

Double staining with anti-rhabas and anti-human IgG followed by quan-
titative analysis of the fluorescence showed a superimposition of the two

signals, which indicated that subepithelial immune deposits were composed

of rhabas and IgG. PLAR was not detected in glomerular immune deposits.

ERT was discontinued in September 2008. Proteinuria progressively de-

creased, but the patient’s clinical condition deteriorated. It was therefore
decided to induce immune tolerance to rhabas by co-administration of

Rituximab, IVlg and methylxenate started in May 2009. ERT was resumed

in July 2009 without inducing a rebound of antibody titer or an increase in

proteinuria. The clinical status again dramatically improved with increasing

doses of rhabas.

Conclusions: We report the first case of rhabas induced allo-immune MN.

The results indicate that deposits were composed of rhabas and anti-rhabas.

The rapid onset of nephrotic syndrome after surgery could result from

anaesthetic-induced alteration of glomerular permeability to rhabas and

anti-rhabas IgG. PLA2R was not detected in glomerular immune deposits.

Double staining with anti-rhabas and anti-human IgG followed by quan-
titative staining was shown by complete inhibition of staining after preincubation

with specific anti-rhabas antibodies. Specific rabbit and sheep anti-rhabas

antibodies were used to detect rhabas in the glomerular deposits.

**OSa062 PATHOLOGICAL PREDICTOR FOR RENAL OUTCOME AND THERAPEUTIC EFFECT: VALIDATION STUDY OF THE OXFORD CLASSIFICATION OF IGA NEPHROPATHY**

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Introduction and Aims: The Oxford classification for IgA nephropathy

(IgAN) had been published recently. In this work we validated the Oxford

classification in a cohort of Chinese population and evaluated the value

of pathology lesions to the therapeutic effect.

Methods: 531 IgAN patients were enrolled in this study. Patients were

divided into 2 groups. 410 patients who met the criteria of the Oxford

Classification (proteinuria >0.5g/day and cGFR<30ml/min/1.73m²) were

fallen into Group 1 and 121 patients who didn’t meet the criteria were

in Group 2. Two groups were analyzed separately. Three pathologists
reviewed the silver-stained sections independently according to the Oxford Classification, and blinded to the clinical information of all the patients.

**Results:** Reproducibility of the pathological lesions was well acceptable among the three pathologists. In Group 1, mesangial hypercellularity, >25% endocapillary hypercellularity, segmental glomerulosclerosis and tubular atrophy/interstitial fibrosis were associated with patients’ initial eGFR, whereas, >25% endocapillary hypercellularity and tubular atrophy/interstitial fibrosis were also associated with initial proteinuria and MAP. However, mesangial hypercellularity and segmental glomerulosclerosis were not significantly associated with initial proteinuria in our cohort. Segmental glomerulosclerosis and tubular atrophy/interstitial fibrosis were independent predictive factors to end stage renal disease (ESRD). In patients of Group 2, none of mesangial hypercellularity, endocapillary hypercellularity, segmental glomerulosclerosis and tubular atrophy/interstitial fibrosis could predict the renal outcome. Patients with mesangial hypercellularity score >0.5 and tubular atrophy/interstitial fibrosis >25% showed less response to RAAS blocker therapy (defined as time average proteinuria <1g/day). Immunosuppressive therapy could provide extra renal protection to patients who showed no response to RAS blocker therapy and patients with endocapillary hypercellularity >25% glomerula involved. Crescent was not found significant in predicting renal outcome and the response to therapy.

**Conclusions:** The reproducibility was well accepted in silver-stained specimens. The Oxford Classification was practical to predict renal outcome in identified patients, but cannot extension for very mild or severe patients. The pathological lesions of the Oxford Classification could provide the clues for immunosuppressive therapy in patients.

**OSa063 5 YEARS FOLLOW-UP RENAL BIOPSIES IN PAEDIATRIC AND ADULT FABRY PATIENTS ON ENZYME REPLACEMENT THERAPY**

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**Introduction and Aims:** There are limited data on the effect of enzyme replacement therapy (ERT) on renal morphology in Fabry patients, and clinical signs are not very sensitive in revealing early changes. The aim of our study was to evaluate the effect of five years ERT on morphologic changes compared with baseline biopsies.

**Methods:** We enrolled seventeen patients (male = 11, female = 6, mean age 30.2±4.5 years, range 7-64 years, 7 patients <18 years) in the study. Different enzyme products and doses were used (agalsidase alpha (AA) 0.2 or 0.4 mg/kg/2w, agalsidase beta (AB) 0.2, 0.5 or 1.0 mg/kg/2w). 70.6% (12/17) patients were treatment naïve at baseline and five patients had been treated with AA 0.2-0.4 mg/kg/2w for 2-3 years. Follow-up renal biopsies were performed after five years or earlier if change of treatment regimen. Two patients (age 53 and 64 years) showed de novo cellular GL3-accumulation. Glomerular sclerosis (GS) stayed absent in all patients on AA/AB 0.2-0.5 mg/kg/2w. No patients developed increased tubular atrophy/interstitial fibrosis or glomerular epithelial cells was found in 23.5% and 29.4% respectively. The podocytes were not completely cleared in any of the patients, but partial clearance was seen in 64.7%; 100% of the patients on AB 0.2-0.5 mg/kg/2w. No patients developed increased GL3-accumulation. Glomerular sclerosis (GS) stayed absent in 35.3% of the patients and disappeared in 35.3%. Increased GS was observed in two older patients (aged 53 and 64 years). No patients showed de novo interstitial fibrosis. Arteriopathy was unchanged in 76.5% and emerged in 23.5% of the patients.

**Conclusions:** In patients with mild and stable nephropathy improvement of Fabry specific renal morphologic changes was seen in different cell types across several ERT dosing regimens. Remarkable reduction of podocyte inclusions were seen in 2/3 of the patients after 5 years ERT, most conspicuous in patients treated with the higher dose of AB. Follow-up renal biopsies are useful in monitoring the adequacy of ERT.

**Disclosure:** The authors have received travel grants from Genzyme and Shire.

**Late breaking clinical trials 1**

**OSa064 ATORVASTATIN AND LOW DENSITY LIPOPROTEIN CHOLESTEROL IN PATIENTS WITH TYPE 2 DIABETES MELLITUS ON HEMODIALYSIS: A POST HOC ANALYSIS OF THE 4D STUDY**

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**Introduction and Aims:** Patients undergoing maintenance hemodialysis are at high cardiovascular risk. Lowering LDL cholesterol with statins has reduced the incidence rate of cardiovascular events. Two randomized, prospective, placebo-controlled trails (4D, AURORA) have been completed in hemodialysis patients which showed no significant effects of statins on cardiovascular outcomes.

**Methods:** We conducted a post hoc analysis of the 4D study (Die Deutsche Diabetes Dialyse Studie) to investigate a) whether LDL cholesterol at baseline is predictive of cardiovascular events and b) whether the effect of atorvastatin on clinical outcomes depends on LDL-C levels at baseline.

**Results:** High levels of LDL cholesterol by trend increased the risks of cardiac endpoints and all-cause mortality. Concordantly, atorvastatin significantly reduced the rates of adverse outcomes in the highest quartile of LDL cholesterol (greater 145 mg/dl or 3.76 mmol/l). The hazard ratios and 95 percent confidence intervals were 0.69 (0.48-1.00) for the composite primary endpoint, 0.58 (0.34-0.99) for cardiac death, 0.62 (0.33-1.17) for non fatal myocardial infarction, 0.68 (0.47-0.98) for all cardiac events combined and 0.72 (0.52-0.99) for death from all causes, respectively. No such decrease was seen in any of the other quartiles of LDL cholesterol at baseline.

**Conclusions:** In patients with type 2 diabetes mellitus undergoing hemodialysis, atorvastatin significantly reduces the risk of cardiac events and death from any cause if pre-treatment LDL cholesterol exceeds 145 mg/dl (3.76 mmol/l).

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