Free Communications

Transplantation 1

SO001 ★ PHYSIOLOGICAL AND MOLECULAR MECHANISMS UNDERLYING THE FUNCTIONAL ADAPTATION OF ADULT-SIZED KIDNEYS TRANSPLANTED INTO PAEDIATRIC RECIPIENTS

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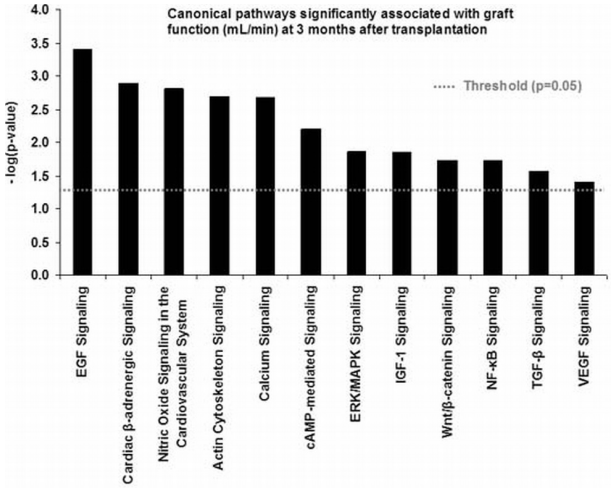
Introduction and Aims: The discrepancy between the vasculature size of small paediatric renal allograft recipients and their adult-sized kidney grafts leads to renal hypoperfusion and decrease in absolute glomerular filtration rate, referred to as functional adaptation of these kidneys to the recipients' size. It was reported recently that this functional adaptation is not completely reversible in the smallest recipients and is associated with irreversible histological damage (interstitial fibrosis, tubular atrophy and tubular microcalcifications), but the underlying mechanisms remain unknown.
Methods: We selected 21 paediatric recipients (age 9.1±6.8 years; range 1.0-21) of an adult-sized kidney who did not have delayed graft function or rejection episodes. Protocol biopsies were obtained at implantation and at 3 months after transplantation and scored according to the Banff classification. Whole-genome expression profiles of the biopsies obtained at 3 months were assessed using Affymetrix cDNA microarrays.
Results: Biopsies obtained at implantation were of pristine histological condition, all but 2 kidney grafts were obtained from living donors (age 32±11 years). Graft function at 3 months after transplantation was highly variable, with an absolute creatinine clearance of 63±23 mL/min (range 31.2-106.1). In concordance with previous studies, absolute creatinine clearance (mL/min) was lowest in the youngest recipients ($p<0.0001$). Using quantitative Significance Analysis of Microarrays (FDR <0.05), 1051 probesets (724 unique identified genes) correlated with creatinine clearance (mL/min). Ingenuity canonical pathway analysis identified significant overrepresentation of relevant and overlapping pathways (Fig. 1; all $p<0.05$) involved in regulation of intracellular Ca^{2+} and in cAMP signaling, important for the regulation of renal vascular tone and blood flow, as well as multiple growth factor signaling pathways. Expression of these overlapping genes correlated significantly with absolute creatinine clearance at 3 months (all q-values <0.05).



Conclusions: The adaptation of adult-sized kidney grafts to the paediatric recipients' size is not only an automatic, passive physiological consequence of low renal blood flow, but is associated with gene expression alterations likely involved in the regulation of renal vascular resistance and GFR autoregulation, which could add to the irreversible tubulointerstitial injury observed in the smallest recipients. In addition to maintaining a hyperdynamic blood flow in the smallest paediatric renal allograft recipients, therapeutic measures targeting the described pathways could further extend the graft survival benefit of adult-sized kidneys transplanted into small children.

SO002 ★ CYCLOSPORIN INDUCES EPITHELIAL TO MESENCHYMAL TRANSITION IN RENAL GRAFTS

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Introduction and Aims: The expression of epithelial to mesenchymal transition (EMT) markers is a reliable predictor of the progression towards interstitial fibrosis and tubular atrophy of the renal grafts. In vitro experiments suggest that calcineurin inhibitors (CNI) can induce EMT of tubular epithelial cells. Although no evidence was ever provided in vivo, this suggests that EMT could be involved in the pathogenesis of renal fibrosis induced by CNI.
Methods: We have previously reported the results of a prospective randomized trial comparing the elimination at month 3 of either cyclosporine (CsA, n=54) or mycophenolate (MMF, n=54) from a triple drug regimen in 108 de novo renal transplant patients. All of them had 2 systematic graft biopsies at months 3 and 12 post engraftment. In the leftover material, we retrospectively detected in tubular cells and by immuno-histochemistry the expression of two validated markers of EMT: the de novo expression of vimentin (Vim) and the cytoplasmic translocation of b-catenin (Cat).
Results: We were able to measure the EMT score at both months 3 and 12 in a total of 68 patients (34 in each group). In the CsA group, the Vim and Cat scores had progressed between 3 and 12 months from 1.530.86 to 1.990.97 (p=0.004) and 1.550.79 to 1.881.04 (p=0.041), respectively, while they remained stable in the MMF group. Remarkably, the 3-month EMT scores were correlated with the 1-year eGFR and the Chronic Allograft Damage Index.
Since acute cellular rejection (ACR) may trigger EMT, we performed a subgroup analysis and excluded the 10 patients who had presented an ACR (CsA group, n=2; MMF group, n=8). Vim and Cat scores respectively progressed from 1.550.86 to 2.00.98 (p=0.007) and from 1.570.79 to 1.891.06 (p=0.065) under CsA, but, strikingly, these scores decreased in patients under MMF and without CsA : Vim from 1.670.92 to 1.380.91 (p=0.13) and Cat from 1.600.85 to 1.260.84 (p=0.047).
Conclusions: First, we confirm that the early expression of EMT markers is of bad prognostic value regarding graft function and histology. Moreover, we found that the EMT score is durably influenced by CsA: maintenance of CsA increases the EMT score, while disruption of CsA attenuates it, as long as the patients dont experience rejection. Thus, in renal grafts, CsA induced fibrosis might involve EMT.

SO003 MESENCHYMAL STEM CELLS PREVENT ACUTE REJECTION IN EXPERIMENTAL RENAL TRANSPLANTATION

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Introduction and Aims: Mesenchymal stem cells (MSC) are multipotent cells that can differentiate into various mature cell types. MSC prefentially home to damaged tissues, where they may contribute to cell regeneration and repair. In addition, MSC have immunomodulatory effects. *In vitro*, MSC inhibit T cell proliferation induced by alloantigens and mitogens and

prevent the development of cytotoxic T cells; *in vivo*, they prolong the survival of experimental skin and cardiac allografts. As yet, the effects of MSC in renal allograft have not been explored. We investigated whether infusion of MSC modifies the course of renal allograft in the rat model.
Methods: Transgenic Sprague-Dawley (SD) rats expressing enhanced green fluorescence protein (EGFP) were used as MSC donors. Syngraft (Lewis RT1 to Lewis RT1, Fisher F344 to Fisher F344) and allograft (Fisher F344 to Lewis RT1) orthotopic kidney transplantations were performed following bilateral nephrectomy. Group A: rats were infused with saline solution in renal artery soon after grafting. Group B: rats were infused with intraarterial $3X10^6$ MSC. No immunosuppressive therapy was administrated. Animals were sacrificed on day 7 and kidney was removed for morphological study. Creatinine clearance was measured on days 0, 3 and 7. Tubulitis and vasculitis were scored according to Banff classification on Movat pentachromic stained renal sections, analysing respectively 150 consecutive tubuli and 10 arteries/section. ED1 and CD8 positive cells were evaluated by immunohistochemistry.
Results: MSC improved renal function in group B vs group A. Tubulitis resulted less heavy in MSC treated (B) than in not treated (A) rats (score % tubuli, T0: B=64.5%, A=36%, $p<0.005$; T3: B=2.6%, A=12.8%, $p<0.0001$). Vasculitis was significantly less severe in group B than in group A (V0: B=50%, A=16%, $p<0.0001$; V3: B=33%, A=70% $p<0.0001$). MSC reduced inflammatory infiltrate; in group B ED1 positive cells number was lower in comparison with A (number of cells per microscopic field B=42.8±8.6, A=78.1±2.1, $p<0.005$) and CD8 positive cells were significantly reduced in group B than in A (B= 14.8±8.4, A=29.4±0.5, $p<0.05$).
Conclusions: Our study demonstrates that MSC infusion significantly attenuates acute renal allograft damage, suggesting that MSC therapy may be an effective strategy to reduce the induction dose of traditional immunosuppressive drugs.

SO004 ATG INDUCTION THERAPY: LONG-TERM IMMUNOLOGICAL EFFECTS 2 YEARS POSTTRANSPLANT

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Introduction and Aims: We showed previously that ATG induction therapy induces a strong decrease of CD4+ T cells together with impaired CD28 expression and in-vitro IL-2 secretion up to 1 year posttransplant. To analyze long-term immunological effects of ATG induction at the 2-year posttransplant time point.
Methods: we used the more sensitive intracellular cytokine analysis instead of ELISA techniques in the same prospective study of 84 renal transplant recipients (ATG, n=44).
Results: ATG induction was associated with an increased frequency of severe infectious disease (20/44 (46%) versus 9/40 (23%) patients, p=0.03) but not CMV disease within 2 years posttransplant. ATG was associated with a persistent decrease of CD4+ T cell counts in peripheral blood ($p<0.0001$) even 2 years posttransplant compared with non-ATG treatment, whereas peripheral blood mononuclear cell counts were no longer diminished. Long-term effects on CD4 cell counts coincided with significantly impaired T cell proliferation (CD69 expression, p=0.01) and intracellular CD4 cell cytokine responses (IL-2, p=0.04 and IL-10, p=0.004). Whereas the diminished IL-10 response was in part counterregulated by a significant increase in IL-10R expression on the subset of CD4+ T cells (p=0.002; no upregulation on CD8+ T cells, B cells and monocytes), we found no increase in IL-2R (CD25) expression. Costimulatory ligand (CD28, CD40, CD40L, CD80, CD86) expression on mononuclear cells was not affected 2 years posttransplant. Interestingly, LPS-stimulated monocyte IL-10 responses were diminished (p=0.04) whereas no long-term effects on IL-4/IL-6 responses, CD4 helper activity or sCD30 levels were found.
Conclusions: Our data show that ATG induction is associated with long-term immunological effects even 2 years posttransplant. Decreased T cell proliferation and CD4 cell IL-2 responses may explain the increased risk of severe infection and lymphoproliferative disease in ATG-treated patients because of inadequate T cell control. The profoundly diminished production of the B-cell growth and differentiation factor IL-10 by CD4 cells may provide previously shown protective effects on graft outcome.
Disclosure: This study was in part supported by the Astellas, Biotest, Fresenius, Novartis and Roche companies.

SO005 PREVALENCE AND MANAGEMENT OF ANEMIA IN RENAL TRANSPLANT RECIPIENTS: DATA FROM 12 EUROPEAN CENTRES

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Introduction and Aims: Although a known cardiovascular risk factor and associated with mortality, anemia in the renal transplant recipients has only recently been receiving an increasing attention. The TRESAM survey found high prevalence of post-transplant anemia in 2003. Here we assess the prevalence and management of post-transplant anemia (PTA) in 2007.
Methods: Our cross-sectional survey was the largest ever done. In our survey laboratory and basic socio-demographic data was obtained from 5834 patients followed at twelve outpatient transplant clinics. Based on the guideline of the American Society of Transplantation anemia was defined as hemoglobin (Hb) less than 130 g/l in males and less than 120 g/l in females.
Results: More than one third (42%) of the patients were anemic. The prevalence of anemia was comparable in males and females. Serum hemoglobin concentration was significantly correlated with the estimated glomerular filtration rate (eGFR) ($r=0.4$, $p<0.001$) and serum ferritin ($R=-0.102$; $p<0.001$). 47% of the patients had ferritin levels lower than 100 ng/ml. In multivariate analysis the estimated glomerular filtration rate, serum ferritin, age, gender, time since transplantation and centers were significantly associated with anemia. An erythropoietin stimulating agent (ESA) was administered to 11% patients. Only 24% of those having Hb less than 110 g/L were treated with an ESA. The prevalence of anemia and also ESA use was significantly different across the participating centers.
Conclusions: Post-transplant anemia is still a prevalent and under-treated condition. The overall prevalence of anemia and the utilization of ESAs in this population appear to be largely unchanged over the last five years. Further studies needed to assess the anemia and ESAs treatment effect of patients' outcomes.

Glomerular diseases

SO006 ★ OUTCOME OF ANCA ASSOCIATED VASCULITIS – LONG-TERM FOLLOW UP OF FOUR RANDOMISED CONTROLLED TRIALS

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Introduction and Aims: Wegener's granulomatosis and microscopic polyangiitis are ANCA associated vasculitides (AAV) with significant

morbidity and mortality. Reports suggest that patients with AAV are at increased risk of malignancies and cardiovascular morbidity and mortality.
Methods: Long-term outcome data on patients of four randomised controlled trials in new onset systemic AAV were collected. The trials included 1 in early systemic vasculitis (NORAM), 2 in patients with mild to moderate renal impairment (CYCAZAREM, CYCLOPS) and 1 in patients with severe renal impairment (MEPEX). A questionnaire was send to the participating physicians and data on survival, renal survival, relapse rate, immunosuppressive therapy, cancer rate, bone fractures and cardiovascular morbidity recorded.
Results: Returns were received from 469 (87.3%) of 537 patients in the original trials. The median duration of follow up was 5.4 years (range 0 to 11.7). There were 132 (28.1%) deaths. The survival at 1, 5 and 10 years were 83.9%, 72.9% and 63.3%. 87 (18.6%) patients required renal replacement therapy. Renal survival at 1, 5 and 10 years was 87.1%, 82.3% and 79.8%. There were 62 new diagnoses of malignant conditions in 52 (11.1%) patients. Of these 31 were solid organ cancers, 9 haematological malignancies and 22 skin cancers. Within 5 years of enrolment 35 (7.6%) patients had a myocardial infarction, 22 (4.8%) a cerebro-vascular accident and 27 (5.9%) a thrombo-embolic event. In addition 38 (8.3%) suffered a bone fracture and 136 (29.6%) developed an infection requiring hospitalisation.
Conclusions: Preliminary analysis suggests that patients with AAV are at increased risk of developing malignant diseases and cardio-vascular events. In addition they suffer a high rate of fractures and severe infections.

SO007 SPECIFIC GENOMIC FINGERPRINTS ARE HIGHLY ASSOCIATED TO THE ONSET AND DEVELOPMENT OF IDIOPATHIC IgA NEPHROPATHY

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Introduction and Aims: Idiopathic IgA Nephropathy (IgAN), the most common biopsy-proven glomerulonephritis worldwide, is characterized by asymptomatic microscopic hematuria (mH) with or without proteinuria or by recurrent episodes of macroscopic hematuria which usually occur in concomitance with mucosal infections of upper respiratory tract or other infections; 30% of patients develop progressive renal failure. Although several investigators have focused on the identification of specific genetic markers associated with the development and progression of this common disease, the genomic and transcriptomic involvement is still not completely defined.
Methods: To this purpose, we measured the intracellular RNA expression level of approximately 15.000 genes, using oligonucleotide microarray chips (HG-U133A, Affymetrix), isolated from whole blood. A large cohort of well characterized IgAN patients [n=16 IgAN patients with persistent mH and normal renal function (IgAN/NORM), n=6 IgAN patients with persistent mH and deteriorated renal function (IgAN/DRF)], and n=7 polycystic kidney disease patients with chronic renal failure (PKD) were included in the study and eight healthy subjects were used as controls (HS). Additionally, in order to identify genes differentially regulated during the acute phase of the disease, we analyzed the genomic profile of 3 patients included in IgAN/NORM group during mH and at the time of the macrohematuria (MH) episode.
Results: Several statistical methods identified 7 genes able to discriminate IgAN/NORM patients from HS (FDR = 43%, $P < 0.0005$), 9 genes highly discriminating IgAN/NORM from IgAN/DRF patients (FDR = 24%, $P < 0.0005$). No genes reached statistical significance in the comparison between IgAN/DRF and PKD patients (FDR>90%). Data analysis showed that genes differentially expressed between IgAN/NORM versus HS were primarily involved in WNT and PI3K/AKT pathways, while those differentiating IgAN/NORM versus IgAN/DRF were implicated in regulatory mechanisms involved in the balance between cell proliferation and apoptosis.
Interestingly, when we analyzed the genomic changes occurring during the MH episodes, we found 62 up-regulated ($p>0.0001$) and 65 down-regulated ($p>0.0001$) genes, respectively, compared to the mH status. These genes represent key elements of important biological processes, such as interferon signaling (e.g., IFIT3, MX1), ubiquitin (e.g. UBE2L6) and immune proteasome (e.g. PSMB8) pathway.
Conclusions: The above innovative high-throughput methodology has shown, for the first time, unlighted biological mechanisms possibly involved in the complex machinery associated to the development and progression of IgAN. In addition, these data may indicate potential targets for screening, prevention and early diagnosis of the disease and more appropriate and effective treatment.

SO008 PERIPHERAL BLOOD AUTOLOGOUS STEM CELL TRANSPLANTATION IN AL AMYLOIDOSIS PATIENTS: THE IMPACT OF RENAL FAILURE

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Introduction and Aims: High-dose chemotherapy followed by peripheral blood autologous stem cell transplantation (ASCT) is presently considered the most effective treatment for AL amyloidosis. Because of the toxicity associated with such therapy, there has been concern about its utility in patients with renal failure and it has been suggested that AL amyloidosis patients with renal involvement are not ideal candidates for myeloablative chemotherapy regimens because of high morbidity and mortality rates.
Methods: We here report our experience with ASCT treating 9 AL amyloidosis patients affected by moderate to severe renal insufficiency and describe the efficacy, the tolerability and the impact on renal involvement of this treatment. The median age of the patients was 50 yrs (4 M/5 F) and monoclonal protein was lambda in 8 cases and kappa in 1 case.
Mean serum creatinine level at presentation was 2.7 mg/dl (range 1.6-9 mg/dl); one patient required chronic hemodialysis since the diagnosis of AL amyloidosis was made. Renal amyloidosis was documented by renal biopsy in all patients and proteinuria was nephrotic in all cases.
Results: Two patients presented cardiac involvement. Seven of 9 patients (77.7%) had a haematological complete response following treatment. Out of the 2 non responder patients: one died and the other is still alive on dialysis, respectively after 8 and 17 months from ASCT. Transplant-related toxicity included grade 2-3 oral mucositis in 3 patients and veno-occlusive disease in 1 patient. The mean follow-up period of the 7 responder still alive patients was 31.2 months. Renal function (GFR) improved from mean 37.4 to mean 74.4 ml/min in all of these cases except 2: one is on chronic hemodialysis and the other presented a severe but stabilized renal failure. Proteinuria decreased from nephrotic levels to less than 0.5 g/24h after over 1 year in 5 patients. One of these patients underwent a second renal biopsy which documented the persistence of glomerular amyloid deposits despite complete remission of proteinuria.
Conclusions: We conclude that ASCT for patients with renal amyloidosis, including those with renal failure, is feasible, tolerable and effective. It results in significant palliation and an improvement in quality of life, particularly in those who achieve a haematological complete response.
Despite complete remission of proteinuria, glomerular amyloid deposits did not regress; a mechanism other than the amyloid mass per se seems to be responsible for the improvement of proteinuria.

SO009 ★ CHANCES OF DIALYSIS INDEPENDENCE AFTER PLASMAEXCHANGE IN RENAL ANCA ASSOCIATED VASCULITIS

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Introduction and Aims: ANCA-associated vasculitis, such as microscopic polyangiitis (MPA), Wegener's granulomatosis (WG) and renal limited vasculitis (RLV) are rare diseases characterized by a rapidly progressive deterioration of renal function with need of dialysis. We investigated the role of PEX in the achievement of renal recovery comparing no PEX (PEX-) and PEX (PEX+) for early dialysis-dependent patients.

Methods: Over 32 years (from April 1975 to October 2007, divided into 3 periods, respectively: 1975-1987, 1988-1997, 1998-2007) we followed up 111 patients (pts), 46 female, 65 male with new diagnosis of primary renal vasculitis biopsy-proven: 35 MPA, 37 RLV and 39 WG. We conducted a single-centre retrospective analysis by assessing clinical presentation, laboratory data, renal histopathology, treatments, patients and renal survival.
Results: The median patient follow up was 57.06 months (mo); 10 patients were lost to follow up after a median of 85.4 mo (range 0.99-211). Median age at presentation was 59±12.7 years, and it was found to increase from 54 yrs during the first, to 59 yrs during the second and to 61 yrs during the third period (p=0.000). Induction treatment was given according to disease severity with plasma exchange (38%), iv cyclophosphamide (23.4%) and iv methilprednisone (71.9%), prednisolone orally, followed by either oral cyclophosphamide or azathioprine. Patient survival was 83.7% at one yr, 63.9% at five yrs and 50.1% at ten yrs of follow up; kidney survival was 69.8% at one yr, 60.6% at two yrs, 57.2% at five yrs, 52% at 10 yrs of follow up. Although both patient and renal survival did not differ between PEX+ and PEX- group on univariate analysis (p=0.234 and p=0.061, respectively), in the first 3 years there was a trend towards a better survival in those patients who underwent PEX as compared with PEX- group. Cox logistic regression pointed out that dialysis (HR 2.242, IC95%1.178-4.267, p=0.014) and older age (>60 yrs) at diagnosis (HR 2.656, IC95% 1.453-4.854, p=0.002) decreased patient survival, while older age at diagnosis appeared to reduce renal survival (HR 2.547, IC 95% 1.498-4.331, p=0.001). Renal recovery has occurred in 6/28 (21%) of the PEX- group and in 9/23 (39%) of the PEX+ group. Univariate analysis showed that iv cyclophosfhamide was associated with better renal recovery (p=0.035).
Conclusions: In the future years the real challenge will be to escape dialysis dependence in elderly patients. Plasma exchange as adjunctive therapy doubles the rate of renal recovery and may reduce immunosuppressive therapy load. An interesting hypothesis-generating study is to shed light on the role of cyclophosphamide pulse in enhancing production of steam cells to repair renal damage.

SO010 PROTEINURIA AS EARLY PREDICTOR OF FINAL OUTCOME IN IgACE, A PLACEBO CONTROLLED TRIAL OF ACE-INHIBITORS IN CHILDREN AND YOUNG PEOPLE WITH IgA NEPHROPATHY AND MODERATE PROTEINURIA

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Introduction and Aims: We recently published (JASN 2007;18:1880-8) the results of an EC Biomedicine and Health Research, double-blind trial involving 23 Centers in Europe, which reported the effect of an Angiotensin converting enzyme inhibitor (ACE-I) in children and young people with IgA Nephropathy (IgAN), proteinuria >1g<3.5 g/day/1.73m^2 and creatinine clearance (CrCl) >50ml/min/1.73m^2. A significant effect of ACE-I was observed for a combined end-point of >30% decrease of CrCl or worsening of proteinuria until ≥3.5g/day/1.73m^2. We are now investigating in greater detail the modification in proteinuria in both treated and placebo patients, looking for its predictive value for final outcome.
Methods: The IgACE trial investigated sixty-six patients, 20.5 (9 –35) years old, randomized to Benazepril, 0.2 mg/kg/day (ACE-I) or placebo (PL) and followed in median for 38 months. The secondary endpoint was a stable proteinuria remission (<0.5g/day/1.73m^2) which was observed in 13/23 (56.5%) ACE-I pts vs 3/34 (8.8%) in PL (P=0.033).
Univariate and multivariate COX regression analysis were performed using categorical and continuous values of proteinuria during the whole follow-up.
Results: Among the treated subjects a mean proteinuria reduction of 40% from baseline was observed. None of them worsened to nephrotic range proteinuria, and the only subject who reached the end point in this group had 30% increase in CrCl. In the placebo group, 7 patients reached nephrotic range proteinuria.
COX regression analysis demonstrated that proteinuria reduction at 12 months was an early predictor of final positive outcome (survival to the combined end point) either when considered as continuous variable [p= 0.002; Exp (B)=1.021 (95 CI 1.008-1.035)] or as categorical achievement of 30% reduction from basal value [p=0.03; Exp (B) 0.095 (95 CI 0.011-0.803).
Moreover proteinuria reduction at 12 months proved predictive of persistent long term proteinuria remission defined as proteinuria < 0.5 g/day/1.73m^2 for at least six months [p= 0.001 for continuous percent reduction; Exp(B)=1.021 (95 CI 1.008-1.035); p=0.01 for categorical 30% reduction; Exp (B)=13.8 (95 CI 3.5-51.8)].
On the opposite, a proteinuria increase at 12 months - either as continuous percent increase or as categorical 30% increase- proved at COX analysis predictive of progression, i.e. reaching of the combined end point [p=0.002 for continuous; Exp (B)= 1.019 (95CI 1.007-1.031); p=0.005 for categorical 30% increase, Exp (B)=12.723 (95CI 2.119-76.384)]
Conclusions: In conclusion, proteinuria modification at 12 months of follow-up in the IgACE trial proved an early predictor of final outcome both in the case of at least a 30% reduction (predicting stable remission), and in case a 30% increase (predicting progression to the combined end-point).

Renal development

SO011 ♦ FUNCTIONAL AND MOLECULAR CHARACTERIZATION OF ADULT RENAL RESIDENT STEM CELLS OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE PATIENTS

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Introduction and Aims: Autosomal dominant polycystic kidney disease (PKD) has been shown to result from loss-of-function mutations of polycystin-1 and polycystin-2. However, the mechanism by which mutations in these genes cause PKD are unknown.
It has been shown that tubular epithelial cells of cystic epithelia present an increased proliferation coupled with a reduced apoptosis and an increased expression of growth factors and their receptors. In addition, cells of cystic epithelia maintain embryonic characteristics. Studies on renal stem cells (SC), that represent the long-term cell population sustaining turn over of tubular epithelial cells, are not available in PKD. We hypothesize that an altered terminal differentiation of PKD-SC leads to increased proliferation and to the maintenance of embryonic phenotype and is involved in cystogenesis. We recently isolated a CD133+ SC population in normal human adult kidney. These cells showed clonogenic and self-renewal ability (Bussolati et al, Am J Pathol 2005).
The aim of this work was to isolate and characterize at a phenotypic and functional level SC from PKD patients, to study the differentiative ability of PKD-derived SC in respect to normal renal SC in vitro and in vivo.
Methods: PKD-SC were isolated from the kidney of 6 patients with PKD, by culture in serum free medium with 1x Insulin-transferrin-selenium, 10-6 M hydrocortisone, 10ng/ml EGF and 2% FCS. PKD-SC cell lines were analyzed by cytofluorimetric analysis and RT-PCR for the expression of stem cell markers and of the renal embryonic markers HOX and Pax-2, in comparison with normal renal SC lines. Growth, clonal ability, resistance to apoptosis were evaluated. Epithelial differentiation of normal SC was evaluated in the presence of FGF-4 and HGF. A murine model of cystogenesis was obtained by implantation of undifferentiated PKD-derived CD133+ renal SC within Matrigel subcutaneously into SCID mice. At day 10, mice were sacrificed and Matrigel plugs recovered and analyzed.
Results: Six different CD133+ cell population were isolated from autosomal polycystic kidneys. Comparing to the normal CD133+ SC population, these cells showed a similar morphology and expression profile but an altered differentiation potential. In particular, this population was not able to fully differentiate in epithelial and endothelial cells in vitro. Moreover, CD133+ PKD-SC cell lines showed an increased apoptosis resistance in respect to normal CD133+ SC. Moreover, when injected subcutaneously in SCID mice in Matrigel, undifferentiated PKD-derived CD133+ SC organized in cystic structures whereas normal CD133+ SC differentiated into tubular structures expressing mature epithelial markers.
Conclusions: The identification of alteration in the phenotypic and functional properties of resident renal SC may provide new insights in the pathogenesis of PKD. Moreover, the development of an in vivo model of human cystogenesis on SCID mice may allow to test drugs interfering

with relevant pathways or to modulate genes or to develop stem cell-based therapies.

SO012 PRONEPHRIC TUBULE DILATATION IN THREE ZEBRAFISH MODELS OF CYSTIC KIDNEY DISEASE IS RELATED TO AN OBSTRUCTION IN THE DISTAL PART OF THE PRONEPHRIC DUCT

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Introduction and Aims: The physiopathology of cystic kidney diseases is still mysterious despite the identification of numerous genes involved. A cilia based model of cyst development recently emerged. In the way to a better understanding of cystic kidney diseases, new animals models beside mice and rats, like C. Elegans or zebrafish are used. The pronephros has been proposed to be a good model. Zebrafish mutants or morphants of genes like *pkd2*,*invs* or *nephrocystin-6* are associated with dilatation of the pronephric tubule. Recently, it had been suggested, in *pkd2* morphant, that dilatation is secondary to obstruction. Our aim is to confirme and understand the obstruction's origine.
Methods: We used a transgenic fish expressing GFP under the control of the *claudin b* promotor. Thus GFP construct is expressed in the zebrafish pronephros. We injected two morpholinos to knock-down the expression of *nephrocystin-6* and *pkd2*. We identified the zebrafish homolog of *Pkdr1*, the gene mutated in han:sprd rat, and we overexpressed it by RNA injection in one cell egg. We realized these experiments in duplicate. More than 60 morphants were observed in live imaging with confocal microscopy. To measure the diameter of the duct and the lumen, we achieved measurement of the distal and proximal part of the duct. We realized a stack of images, we kept the image where the diameter is the wider and made three measures. The diameter of this part of the pronephros for one fish is the mean of the three measurments.
Results: We determined the normal morphology of pronephros in live, non fixed normal zebrafish. We established the normal pronephros dimensions (40 fishes) at 24 hours post fertilization (hpf), 48 hpf and 72 hpf. The diameter of the pronephros at 24 hpf is 20±2 μm and the lumen diameter is 2±1.1 μ. There is a small increase in the diameter during development. In *Nephrocystin-6* and *Pkd2* morphants, beside the previously described anomalies like abnormal axis or hydrocephalus, we observed that tubular dilatation begins after 36 hpf. Before, the size of the pronephros is only slighty and non significantly increased. By three days, we observed in more than 70% of morphants a huge tubular dilatation. The diameter of the proximal part of the pronephros is more the double that normaly observed, and the lumen diameter is more than ten times the normal one. The pronephros duct dilatation is extended to the 18th somites. At this part we observed the destruction of the normal tubule architecture, with disappearance of the pronephic duct lumen. We observed, only in this part of the duct, an apoptosis of the duct cells. This process is begining after 24 hpf. In the *Pkdr1* overexpressing fishes we observed a tubular dilatation begining before the 24 hpf. In this case the dilatation is associated with a malformated cloaca.
Conclusions: We demonstrated using live imaging microscopy that pronephric tubular dilatation in three models of cystic kidney disease is secondary to an obstruction of the distal part of the pronephric duct. We propose a "come back" of the obstructive theory of cystic kidney diseases at least in zebrafish.

SO013 ★ EVEROLIMUS PULSE TREATMENT HALTS POLYCYSTIC KIDNEY DISEASE PROGRESSION LONG-LASTING IN Cy/+ RAT

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Introduction and Aims: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by dysregulated tubular epithelial cell (TEC) growth. The mammalian target of rapamycin (mTOR), a key controller of cell growth and proliferation, is aberrantly activated in TECs of cystic kidneys. Continuous treatment with specific mTOR inhibitors retards rodent polycystic kidney disease. Here we examined the effect of pulse administration of the specific mTOR inhibitor everolimus on cystic kidney disease progression in the Han:SPRD rat, a model which resembles phenotypically human ADPKD.
Methods: Four-week-old male heterozygous cystic (Cy/+) and wild-type normal (+/+) rats were administered everolimus or vehicle (3 mg/kg/day) once daily by gavage feeding for 12 weeks (continuous treatment). In a different schedule the drug was administrated for 5 weeks followed by 7 weeks without treatment (pulse treatment). BUN, creatinine and everolimus blood trough levels were monitored throughout. At week 16 the rats were sacrificed and kidney weights were measured to calculate the ratio of 2 kidneys/total body weight (2K/TBW). Cyst volume density was measured and the ratio of cyst volume to total cortex area was determined. Glomerular tuft areas were also measured and the glomerular volume (GV) was subsequently estimated.
Results: A 5-week pulse everolimus treatment resulted in a long-lasting decrease in cystogenesis with preservation of kidney function (BUN, creatinine) similar to continuous everolimus treatment. Cyst volume density was reduced by 76% ($p < 0.01$) with pulse treatment and by 54% ($p < 0.05$) with continuous treatment. Interestingly, after the pulse everolimus treatment an increase of kidney weight and an enlargement of glomerular volume was seen. 2K/TBW and GV increased by 36% ($p < 0.05$) and 125% ($p < 0.05$) in pulse vs. continuous everolimus-treated Cy/+ rats.
Conclusions: A 5-week pulse therapy with everolimus had a long-lasting inhibitory effect on cystogenesis which was accompanied by secondary glomerular hypertrophy after stopping the drug in Cy/+ rats. Continuous everolimus treatment may protect the glomeruli by preventing secondary glomerular hypertrophy.

SO014 ❋ THE *Pkhd1* COMPLEX TRANSCRIPTIONAL MECHANISM GIVES RISE TO ALTERNATIVE TRANSCRIPTS WITH PRESERVED OPEN READING FRAME, A PATTERN DISRUPTED BY A FRAMESHIFT MUTATION.

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Introduction and Aims: Mutations in the *PKHD1* gene are responsible for all typical forms of autosomal recessive polycystic kidney disease (ARPKD). Both *PKHD1* and *Pkhd1*, its mouse ortholog, are associated with a complex splicing pattern that yields a high number of alternative transcripts. Gene-targeted mouse models suggest that *Pkhd1* is functionally divergent in kidney and liver and that distinct transcripts are associated with specific roles. To identify and characterize key transcripts and their potential functions, we have examined the transcriptional profile of this gene in kidney and liver, in wild type (WT) and *Pkhd1*$^{cl/cl}$ mice. The cl spontaneous mutation occurs in exon 48, leading to a frameshift (c.7589delGGinsT). Homozygous mutants develop biliary dysgenesis and cystic liver disease by 1 month of age, but no morphological evidence of renal abnormalities.
Methods: Long- and short-range RT-PCR were used to amplify transcripts from both mouse tissues, using a large set of primer pairs. Products representative of distinct transcripts are being subcloned and sequenced.
Results: Experiments in WT kidneys and livers revealed different transcript profiles between the two organs. Comparative analysis of WTs and MUTs, in turn, showed specific isoform patterns for both kidney and liver in the MUTs. The systematic characterization of the products is allowing us to develop a catalog of organ-specific and disease-related transcripts. To date, we have analyzed more than 100 products amplified from kidney cDNAs with primers positioned in exons 1 and 67. Among the 24 transcripts fully-characterized thus far, 12 represent 6 transcripts expressed in both WT and MUT kidneys, while 5 were amplified only in WTs and 7 were specific to the MUTs. All 11 transcripts isolated from WTs appear to preserve the aminoacid sequence of the *Pkhd1* longest open reading frame (ORF), 7 of them using the original translation start site (TSS). In contrast, 3 of the 7 MUT-specific transcripts likely do not preserve this ORF, and only

3 MUT-specific transcripts use the original TSS. Prediction scores for the acceptor and donor sites of all transcripts (with a single donor site exception) were >0.70. Analyses of liver WT and MUT transcripts, as well as products amplified with other strategic primer pairs, are in progress. Lastly, in situ hybridization experiments are in progress to confirm key observations at the tissue level.

Conclusions: Taken together, our data demonstrate that *Pkhd1* undergoes tissue-specific alternative splicing and suggest that the transcriptional processing is altered in the mutant gene. Moreover, the ORF analyses suggest that most of the WT alternative transcripts are likely functional, while several of the newly generated MUT transcripts may encode significantly shorter related proteins, or be non-functional. We conclude that the integrity of *Pkhd1* is essential for transcriptional processing and dysfunctional splicing may be involved in ARPKD pathogenesis.

SO015 ★ DISEASES LINKED TO *TCF2*/HNF-1b GENE ANOMALIES: FROM FOETUS TO ADULT

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Introduction and Aims: Hepatocyte nuclear factor-1 beta (HNF-1b), encoded by the *TCF2* gene, is responsible for maturity-onset diabetes of the young type 5 and developmental nephropathies.

Objectives: To describe clinical spectrum, natural history and genetic findings of diseases linked to *TCF*2 gene anomalies from foetal life to adulthood.

Methods: Multicenter, descriptive study: 35 patients with *TCF*2 gene anomalies, 33 prenatally diagnosed including one *in utero* death, 2 postnatally diagnosed.

Annual standardized clinical, radiological and biochemical evaluation.

Results: Thirty three patients were prenatally diagnosed with developmental nephropathies: 18 foetuses had bilateral hyperechogenic kidneys, 10 had hyperechogenic kidneys with controlateral multicystic dysplastic kidney (MCDK), 2 had unilateral multicystic kidneys, one foetus had bilateral renal hypoplasia, one had renal agenesis with controlateral hypoplasia and one had non viable bilateral MCDK. Twenty one foetuses had cysts (63.6%). The mean prenatal renal length was 0.29±1.45 SD. After birth, renal growth was impaired conducting to small kidneys with a mean postnatal renal size of -0.93±1.66 SD. In patients with a solitary kidney there was no compensatory hypertrophy. At the last visit, 28 patients had cysts (82%), mainly cortical microcysts (23 patients). After a mean follow-up of 90.65±80.4 months, 33 patients demonstrated bilateral nephropathies and 1 patient had unilateral MCDK. The mean glomerular filtration rate (GFR) was 77.8±32.9 ml/min/1.73m^2. Seventeen patients had a GFR ≥ 90 ml/min, six a GFR of 89 to 60 ml/min, seven a GFR of 59 to 30 ml/min, two a GFR of 29 to 15 ml/min and two patients had end stage renal failure. The mean annual decline of the filtration glomerular rate was 3.4 ml/min/year

Extra renal manifestations included diabetes, hyperuricaemia, cholestasis, exocrine pancreatic insufficiency with low faecal elastase and asymptomatic pancreatic hypoplasia.

The main *TCF2* gene anomaly was a complete heterozygous deletion (20/35 patients). We found 10 different heterozygous point mutations: 4 missense mutations (p.G76C in 3 patients, p.Q253P in two patients of the same family, p.G285D, p.N289D), 3 splicing mutations (c.IVS1-1G>A, c.IVS1+6C>T, c.IVS6+ 1G>A), 1 nonsense mutation (p.Q454X), 1 frameshifting mutation (c.717delG), 1 involving the initiation codon (p.M1 or c.3G>A in two twins), and 1 single-exon deletion (deletion of exon 4).

Sixty six percent of the genetic anomalies appeared *de novo.*

Conclusions: *TCF*2 gene anomalies are associated with variable post-natal renal and non renal phenotypes. Therefore, close post-natal follow-up of patients with *TCF2* gene anomalies is strongly recommended.

Acute kidney injury 1

SO016 ❁ ♦ URINARY MIDKINE IS A SENSITIVE BIOMARKER OF ACUTE TUBULAR NECROSIS IN HUMAN

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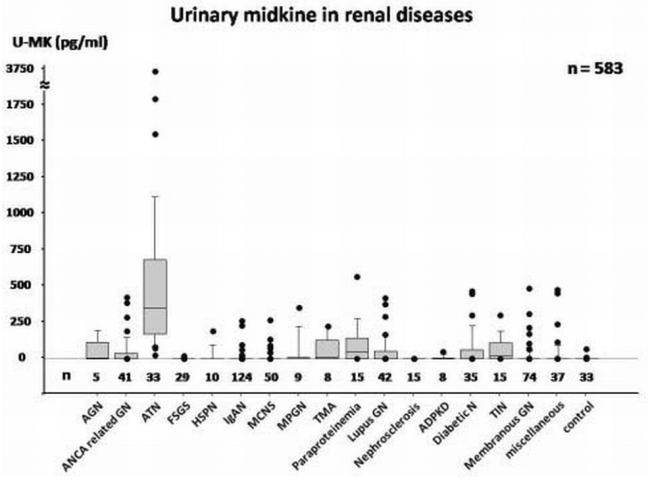
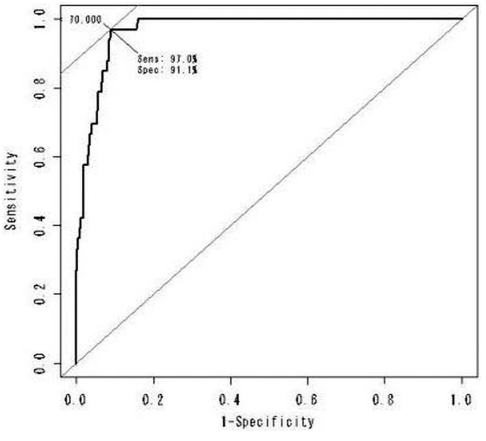
Introduction and Aims: Midkine (MK) is a heparin-binding growth factor, and promotes chemotaxis of leukocytes in the animal models of an ischemic renal reperfusion injury (Sato W. et al., J Immunol 2001; 167: 3463-3469) and a diabetic nephropathy (Kosugi T et al., Am J Pathol 2006; 168; 9-19). The enhanced expression of MK was detected in the proximal tubules after ischemic injuries. Here, we evaluated whether human MK is shed into urine from the tubular epithelial cells, and may serve as a potent urinary biomarker of acute kidney injuries in human.

Methods: Cultured human tubular epithelial (HK2) cells were treated with hypoxia (1% O_2+5%CO_2) or 20 μM BSA. Cell lysates and culture media were subjected for western blot analysis. For the measurement of urinary MK, 583 patients, including controls (n=33), patients with different forms of acute tubular necrosis (ATN) (n=33), and patients with other chronic renal diseases (n=517), were studied. Urinary MK level was determined by enzyme-linked immunoassay.

Results: Western blot analysis showed that the increased expression of MK was detected in the culture media from HK2 cells treated with hypoxia, but not from HK2 cells treated with 20 μM BSA. There were no differences in the MK expression in the cell lysates.

The urinary MK levels were significantly higher in patients with ATN





(546.4±695.7, n=33) compared to levels in patients with other chronic renal diseases (26.9±80.2, n=516) or controls (2.1±11.0, n=33). For concentration in urine of MK, the receiver-operating characteristic (ROC) curve analysis for diagnosis of ATN showed that sensitivity was 97.0%, and specificity was 91.1% for a cut-off value of 70.0 pg/ml.
Conclusions: A shedding of human MK was detected in the urine from the patients with ATN. Furthermore, urinary MK level may serve as a sensitive biomarker of ATN facilitating the early diagnosis of the disease.

SO017 FACTORS AFFECTING POST-HEART SURGERY RENAL FUNCTION: A LARGE ITALIAN DATABASE

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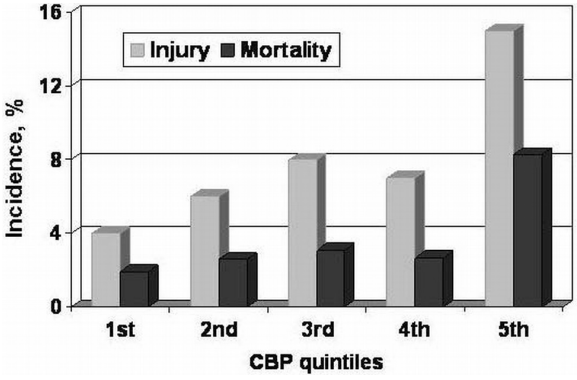
Introduction and Aims: Heart surgery procedures, especially if open heart, may affect renal function. Previous works have reported that the duration of cardiopulmonary bypass (CBP) is a risk factor for acute kidney injury.
The National Cardioanethesia DataBase collects data from 14 Italian cardiosurgery intensive care units (ICU). Each case record form collects about 170 demographic and peri-operative variables, from entry to discharge.
Methods: We have analysed the database in order to identify peri-operative predictors of severe kidney damage, expressed by two primary outcomes: *Injury (*creatinine doubling and/or renal replacement therapy -RRT- need) and *Failure* (need of RRT). A special focus was made on the role of the CBP time.
A total of 11,681 records were analysed: 11,092 CBP procedures and 589 off-pump. Data processing included descriptive statistics (Student's T test and c^2 test), univariate and multivariate analyses.
Results: The incidence of kidney *injury* was 7.2%, *failure* 2.7%. Both increased significantly the ICU as well as the hospital stay ($p<0.0001$, Mann-Whitney test). On-pump procedures were followed by *Failure* and RRT slightly, but significantly more often than off-pump ones (1.84 vs. 1.02%, p=0.007).
Mortality was significantly greater in *failure* (43.1%) than *injury* (26.2%, $p<0.05$).
Subdividing the CBP time in quintiles (<53 min, 54-71, 72-92, 93-122 and 123-591 min) the incidence of *injury* and death rate were similar up to the 4th quintile and acutely increased only in the last quintile. Even with the univariate analysis, the odds ratio (OR) for *injury* risk was stable up to 122 min (1.76, 1.81, 1.84), while it was 3.84 for the 5th CBP quintile (> 122 min). Most relevant risk factors, both pre- and post-surgery, were sepsis (OR 37), low cardiac output (19.7), intra-aortic balloon pump (16), shock (8.09), need for inotropes (6.99), ejection fraction <30% (5.6), pre-operative creatinine (3.87 per 1 mg increase), previous myocardial infarction (3.8).
Finally, at the multivariate analyses, the CBP time quintiles, both for *injury* and *failure,* did not turn out to be significant, and the presence of sepsis (OR 5.49), low cardiac output states (5.3), pulmonary dysfunction (2.8) and pre-operative creatinine (2.3) remained the most relevant risk factors.



Conclusions: To conclude, the responsibility of the CBP time in itself in kidney damage seems lower than expected on the basis of the literature data, at least for a large duration range (<122 min). On the contrary, in most cases the occurrence of kidney damage is the result of the peri-operative clinical complications or prior kidney dysfunction.

SO018 ★ SHIGA TOXINS BOUND TO POLYMORPHONUCLEAR LEUKOCYTES (Stxs-PMN): CLINICAL OBSERVATIONS IN CHILDREN WITH HEMOLYTIC UREMIC SYNDROME (HUS)

Laura Fuiano[1], Carmine Pecoraro[1], Maurizio Brigotti[2], Pier Luigi Tazzari[3], Elisa Ravanelli[2], Domenica Carnicelli[2], Stefania Barbieri[2], Gaia Scavia[4], Fabio Minelli[4], Francesca Ricci[3], Andrea Bontadini[3], Roberta Alfieri[5], Pier Giorgio Petronini[5], Alfonso Ferretti[1], Carmine Pecoraro[1], Fabio Paglialonga[6], Alberto Edefonti[6], Maria Antonietta Procaccino[7], Alberto Tozzi[7], Alfredo Caprioli[4]. *[1]NephroUrology, Santobono Children's Hospital, Naples, Italy; [2]Experimental Pathology, University of Bologna, Bologna, Italy; [3]Department of Immunohematology, Sant'Orsola-Malpighi Hospital, Bologna, Italy; [4]Istituto Superiore di Sanità, Rome, Italy; [5]Department of Experimental Medicine, University of Parma, Parma, Italy; [6]Pediatric Clinics "De Marchi", Milan, Italy; [7]Bambino Gesù Hospital, Italy*

Introduction and Aims: Intestinal infections by Stxs-producing *Escherichia coli* (STEC) cause childhood HUS. Stxs released in the gut are targeted to renal endothelium after binding to PMN. Stxs-PMN can be detected by specific antibodies and flow cytometric techniques and this test can be exploited for early diagnosis of STEC infection in HUS. In this study relationships between main clinical features of HUS children and the amounts of the Stxs-PMN were established.
Methods: Laboratory tests for STEC infection (STEC isolation and presence of free Stxs in feces, serum levels of antibodies to LPS of five major STEC serogroups (O157,O26, O103, O111, O145) and Stxs-PMN were performed in 39 children with HUS D+ (15 males, median age 26 months).
Results: Any evidence of STEC infection was present in 35 pts (89.7%); Stxs-PMN were detected in 18/39 (46.2%) that were divided into two groups according to Mean Channel Value of Fluorescence (MCV) that represents a parameter to quantify the amount of Stxs-PMN: Group 1 (MCV $\geq$ 1.5) high Stxs level, 6 cases; Group 2 (MCV < 1.5) low Stxs level, 12 cases. The median time interval between the sampling of PMN and the diagnosis of HUS was similar for the two groups: 7 and 8 days, respectively. The two Groups were compared for platelet and neutrophils counts, hemoglobin and serum creatinine levels, need for dialysis and/or transfusions and neurological complications. No significant differences in the trends of platelet and neutrophil counts were observed in any of the Groups. Conversely, serum creatinine was normal in Group 1, whereas it was increased in Group 2 during the whole period of observation. Accordingly, the percentage of patients needing dialysis was lower in Group 1 (30%) and also the duration of the dialytic treatment was significantly shorter (3.5±2.1 days) in Group 1 than in the Group 2 (50% and 12.8±5.8, respectively). By contrast neurological complications were more frequent in Group 1 than in Group 2.
Conclusions: Our data suggest that HUS children with high circulating Stxs-PMN levels show more frequently neurological complications whereas the severity of renal involvement is higher in low-toxin patients. Our conclusion is that a self-amplifying circle triggered by low doses of toxins leads to the production of pro-inflammatory mediators of renal damage, while neurological complications are more likely due to sudden brain endothelial injuries imposed by large amount of toxins.

SO019 FREE LIGHT CHAIN REMOVAL HEMODIALYSIS INCREASES RENAL RECOVERY RATE AND IMPROVES PATIENT SURVIVAL IN PATIENTS WITH CAST NEPHROPATHY

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Introduction and Aims: High cut-off hemodialysis (HCO-HD) effectively

removes large quantities of serum free light chains (FLC) in multiple myeloma patients with cast nephropathy. Whether this translates to sustained reductions in serum FLC concentrations and improved patient outcome is undetermined. This study assessed the combination of HCO-HD and chemotherapy as a therapeutic strategy to rapidly reduce serum FLC concentrations in patients with multiple myeloma, dialysis dependent renal failure and cast nephropathy.

Methods: Standard chemotherapy regimens were initiated in all patients. HCO-HD was undertaken using two Gambro HCO 1100 dialysers in series, for 8 hours daily for the first 5 days. Then 8 hours on alternate days through to 21 days. Blood and dialysate flow rates were 250ml/min and 500ml/min, respectively. Dialysis independence and patient survival in 17 patients treated with HCO-HD were compared with a case matched historical control population treated with standard high flux dialysis (n=17).

Results: HCO-HD resulted in sustained reductions in serum FLC concentrations in 12 of 17 patients (median 86% (range 50-93)). These 12 patients became independent of dialysis at a median of 27 days (range 13-50). Five patients had chemotherapy stopped because of early infective complications and did not achieve sustained reductions in serum FLCs. These patients did not recover renal function and had a significantly reduced survival ($P<0.002$). Only two of 18 (11%) control patients became independent of dialysis. Figure 1 - rates of independence of dialysis were significantly greater in study patients (solid line) than controls (broken line), $P<0.001$. In both groups, patients with cast nephropathy who recovered renal function (solid line) had a significantly improved survival compared with those who remained dialysis dependent (broken line), $P<0.012$ (figure 2).

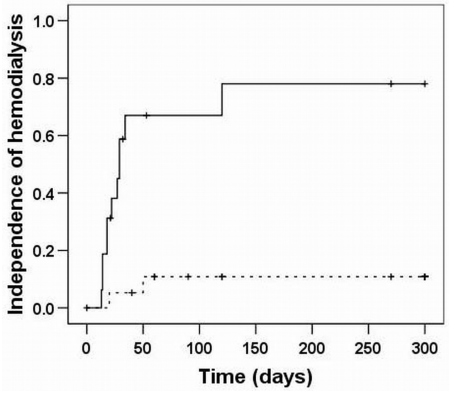

Figure 1

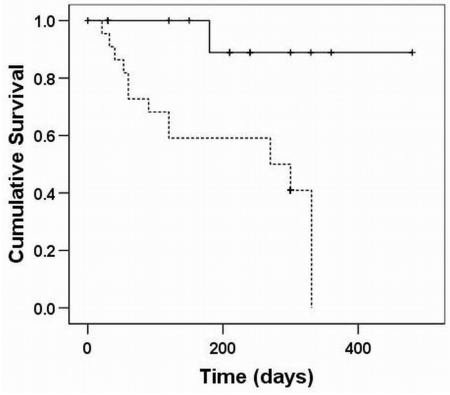

Figure 2

Conclusions: In conclusion, extended HCO-HD resulted in sustained reductions in serum FLC concentrations in patients with cast nephropathy. This was associated with an increased rate of renal recovery and improved survival.

Diabetes 1

SO020 THE IMPACT OF DIABETES-SPECIFIC PROCESSES OF CARE (POC) ON CLINICAL OUTCOMES OF DIABETIC HEMODIALYSIS (HD) PATIENTS: THE DIALYSIS OUTCOMES AND PRACTICE PATTERNS STUDY (DOPPS)

S.P.B. Ramirez[1], J. Thumma[1], B.M. Robinson[1], F. Tentori[1], M. Krishnan[2], C. Combe[3], H. Rayner[4], R.L. Pisoni[1]. *[1]Arbor Research Collaborative for Health, Ann Arbor, MI, USA; [2]Amgen, Thousand Oaks, CA, USA; [3]Centre Hospitalier Universitaire de Bordeaux, Bordeaux, France; [4]Birmingham Heartlands Hospital, Birmingham, United Kingdom*

Introduction and Aims: The impact of diabetes mellitus (DM)-specific practices on clinical outcomes of diabetic patients (pts.) on HD has not been studied. The goal of this study is to identify whether DM-specific practices can modify the risk for adverse clinical outcomes in these pts.

Methods: Data are from DOPPS III, a prospective observational study of HD pts. randomly selected from representative dialysis facilities in 12 countries. Descriptive statistics of 1,784 prevalent diabetic pts. from 177 facilities were examined. A DM POC questionnaire was completed by each facility's medical director. Cox models were used to investigate mortality risk by facility DM POC. Models were adjusted for age, sex, race, time on HD, 13 comorbidities, Hgb, serum ferritin, serum creatinine, serum albumin, and region, while accounting for facility clustering. A DM management score (DMS) was developed based on the reported frequency of foot exams, HbA1c measurements, and cardiovascular disease screening.

Results: At baseline, 52.6%, 41.6%, 15.2%, and 46.6% had coronary artery disease, peripheral vascular disease, diabetic gastroparesis, and diabetic retinopathy, respectively. Large facility variation for DM POC was noted.

Absence of routine DM practices was associated with significantly increased mortality risk. Pts. from facilities without routine practices for foot exam or for HbA1c monitoring had significantly higher mortality risk when practices were modeled independently or with all three practices in one model (Table 1). DM care provided by either an endocrinologist or nephrologist as compared to a PCP when modeled independently was associated with a significantly lower mortality risk (RR:0.6, p=0.015 and RR:0.7, p=0.046, respectively).

Because multifaceted care is most effective for the management of DM, facility DMS was created by combining DM management practices. Progressively lower facility DMS was significantly associated with progressively increasing mortality risk. A score of 2 or below, characterized by less frequent DM POC, had a RR of 1.53 (p=0.02). These findings suggest the importance of fulfilling guideline recommendations since even a reduction in frequency of one of the practices can significantly increase the risk of mortality.

Table 1: All-Cause Mortality Association with Diabetes Practice

Practice Pattern	Frequency of Practice	Facilities (%)	Adjusted Risk Ratio (RR)	
			Practices modeled separately (RR)	Practices modeled together (RR)
Foot Exam	Every 1-2 months	44	1.00	1.00
	3-4 mo to yearly	24	1.34	1.23
	No routine	32	**1.57***	**1.44***
HbA1c Measurement	Every 1-2 months	38	1.00	1.00
	3-4 mo to yearly	60	1.40	1.12
	No routine	2	**2.07***	**2.03***
Screen for CVD	Every 1-2 to 6 months	24	0.77	0.90
	Yearly	35	1.00	1.00
	No routine	41	1.01	1.02

*p<0.05

Note: Other DM practices (fasting lipid profile, retinal exam, review of dosage, use of DM-specific medications and physician-patient-dietitian DM-specific discussion) did not have significantly higher risk for all-cause mortality.

Conclusions: Our analyses demonstrate that 1) large variations exist in the delivery of DM POC to the diabetic ESRD population, 2) facilities with DM management practices tend to have lower mortality rates compared to those that do not, and 3) the DMS developed from these findings requires validation in other HD settings.

Disclosure: The DOPPS is supported by research grants from Amgen, Inc. and Kirin Pharma Co., Ltd., without restrictions on publications.

SO021 EPOETIN DELTA TREATMENT CORRECTS NERVE BLOOD FLOW AND FUNCTION IN DIABETIC RATS: RELEVANCE FOR VASCULAR COMPLICATIONS AND NEUROPATHY

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Introduction and Aims: In humans, diabetes is a major risk factor for chronic kidney disease (CKD). Anaemia is a common complication of CKD; it reduces quality of life and can exacerbate macrovascular and microvascular complications of diabetes. Erythropoiesis-stimulating agents (ESAs) have been shown to be effective in correcting anaemia, but can also have pleiotropic effects that could produce additional benefits in diabetes. We examined the effects of epoetin delta (Dynepo®, Shire plc), a human-cell-derived ESA, on measures of nerve blood flow, nerve function and erythropoiesis in diabetic rats.
Methods: Studies used Sprague-Dawley rats. Streptozotocin-induced diabetes was left untreated for 4 weeks, followed by 4 weeks of epoetin delta treatment. Study 1 was dose-ranging and measured motor nerve conduction velocity (NCV). Rats were treated with epoetin delta 21–1667 IU/kg, once weekly (QW) or thrice weekly (TIW). In study 2, epoetin delta doses of 150 IU/kg (QW or TIW) or 1500 IU/kg (QW) were used for in-depth analysis of nerve perfusion and function. In unanaesthetized rats, tactile allodynia (tested by foot withdrawal reflexes to von Frey hair stimulation) and thermal hyperalgesia (tested by noxious heat stimulation) were studied. Under thiobutabarbital anaesthesia, sciatic nerve nutritive endoneurial blood flow was assessed by hydrogen-sensitive microelectrode polarography, followed by measurement of motor NCV in the sciatic nerve and sensory NCV in the saphenous nerve. Blood samples were analysed for haematopoietic effects.
Results: In study 1, diabetes reduced motor NCV by 21%; this was corrected by epoetin delta treatment. ED_{50} values were similar for QW and TIW administration (mean ± SEM: 94.7±1.2 IU/kg and 54.1±1.3 IU/kg, respectively). In study 2, a 53.7±4.2% reduction in sciatic nerve blood flow was corrected by epoetin delta (49.2±10.1% [150 IU/kg, TIW]; 50.0±7.0% [150 IU/kg, QW]; and 74.3±4.6% [1500 IU/kg, QW]). Diabetic rats showed tactile allodynia (threshold for foot withdrawal on stimulation reduced by 58%) and thermal hyperalgesia (foot withdrawal latency decreased by 24%). Allodynia was partially corrected (69%) by epoetin delta 150 IU/kg (QW or TIW) and completely corrected by 1500 IU/kg (QW). Similarly, thermal hyperalgesia was partially (64%; 150 IU/kg, QW or TIW) or completely (1500 IU/kg, QW) corrected. A 16% sensory NCV deficit was largely (84%) corrected by all doses. In both studies, haematocrit (Hct), haemoglobin (Hb), and red cell and reticulocyte counts were unaffected by diabetes or QW treatment. For TIW treatment, Hct, Hb and red cell count were elevated at the highest dose (1667 IU/kg) and reticulocyte count was increased at doses ≥ 186 IU/kg.
Conclusions: We have shown that epoetin delta has a marked positive effect on nerve blood flow in diabetic rats, which was reflected by improved neural function. This occurred even at epoetin delta doses that did not promote haematopoiesis, representing a pleiotropic vascular effect of epoetin delta treatment that could be beneficial for the vascular complications of diabetes.
Disclosure: This study was supported by a research grant from Shire plc.

SO022 ★ DECREASED ENDOTHELIAL PROGENITOR CELLS IN TYPE 2 DIABETIC PATIENTS WITH MICROALBUMINURIA OR MACROALBUMINURIA

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Introduction and Aims: It is generally accepted, that type 2 diabetic patients presenting with microalbuminuria (Mi-A) or macroalbuminuria (Ma-A) have an increased risk for cardiovascular morbidity and mortality, although the underlying mechanism are not fully explained. Endothelial progenitor cells (EPC) are bone marrow derived cells involved in adult neovascularisation and endothelial homeostasis. Previous studies have shown that low EPC in peripheral blood may predict macrovascular disease and mortality in nondiabetic patients with high cardiovascular risk. Thus, it was of interest to investigate the potential role of EPC in type 2 diabetic patients presenting with Mi-A or Ma-A in comparison with normoalbuminuric type 2 diabetic patients.
Methods: In total, 85 patients with type 2 diabetes were included into the study: 45 with normoalbuminuria (No-A), 25 with Mi-A and 15 with Ma-A. The patients in the three groups were carefully matched and did not differ (by ANOVA) for the following: age (62.8±10.4 years), HbA1c (8.2±1.7 rel.%), BMI (30.0±5.2 kg/m^2), systolic and diastolic blood pressure (144±21 and 83±13 mm Hg), total cholesterol (184±42 mg/dl), LDL-cholesterol (96±34 mg/dl), HDL-cholesterol (47±12 mg/dl) and triglyceride (223±159 mg/dl). Circulating progenitor cells (CPC; CD34+/133+), EPC (CD34+/133+/309+) and activated EPC (CD34+/133+/309+/31+) were enumerated by flow cytometry in peripheral blood.
Results: EPC were decreased in patients presenting with Mi-A compared with patients with No-A (102±74 vs 142±79, p=0.04). In patients with Ma-A the number of EPC was even more decreased (55±32 vs 142±79; p<0.001). Patients with Mi-A or Ma-A were also significantly different for the number of EPC (102±74 vs 55±32, p=0.01). By contrast, circulating progenitor cells, which have an important role in the haematopoietic system, were not significantly different among the 3 groups of type 2 diabetic patients with No-A, Mi-A or Ma-A (p=0.37).
Conclusions: In conclusion, this is the first study demonstrating decreased numbers of endothelial progenitor cells in type 2 diabetic patients with microalbuminuria or macroalbuminuria. Since low EPC are important predictors of future cardiovascular morbidity and mortality in nondiabetic high risk patients, these new findings could be relevant for the understanding of the high cardiovascular risk of type 2 diabetic patients with microalbuminuria or macroalbuminuria.

SO023 ★ OVERT TYPE 1 DIABETIC NEPHROPATHY: EFFECT OF ADD2 AND ACE POLYMORPHYSMS ON PROGRESSION TOWARDS END STAGE RENAL DISEASE

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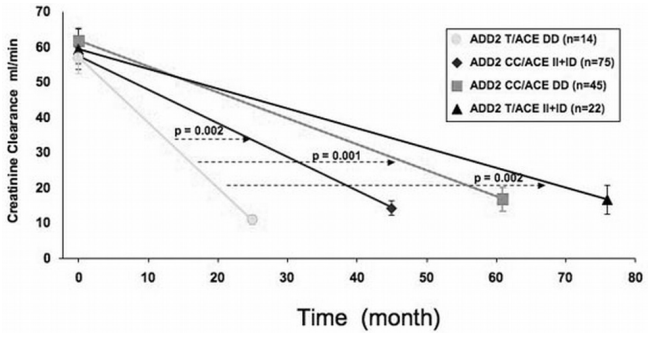
Introduction and Aims: Diabetic nephropathy (T1DN) and the associated renal failure account for the reduced life expectancy of patients with type 1 diabetes (T1DM). Although almost all patients with T1DM are affected by diabetic retinopathy over time, only 25 to 35% of these patients develop DN. Chronic hyperglycemia cannot fully account for the pathogenesis of renal complications. Genetic factors play an important role, as shown by familial studies. Angiotensin-converting enzyme (ACE) and the adducin (ADD1, ADD2, and ADD3) gene polymorphisms are associated with cardiovascular and DN risk factors, respectively. In a retrospective population study and in cell models, we investigated the combined effects of these polymorphisms.
Methods: We randomly recruited 251 T1DM without DN and 141 T1DN with a mean follow-up of 5 years. All patients were genotyped for ADD1, ADD2, ADD3, and ACE polymorphisms. To clarify our epidemiological observations among the genotype studied, we investigated the combined effects of ADD2 and ACE on the membrane-bound ACE activity in fibroblasts.
Results: Hardy-Weinberg equilibrium was respected for all polymorphisms studied. No significant differences were present in genotypes frequency between DN and control patients. T1DN carrying ADD2 T/ACE DD



reached dialysis 50 months before than those carrying ADD2 T/ACE II-ID (ADD2*ACE Interaction p =0.009 after correction for confounders). Furthermore, in fibroblasts carrying the ADD2 T/ACE II+ID the membrane-bound ACE activity increased in T1DN (6.22±1.1 nanomoles of generated hippuric acid per milligram of protein per minute) than in control (3.74±0.7; p=0.09)

Conclusions: The ADD2 permissive effect on ACE polymorphisms may have a determinant role in the progression toward end stage renal disease in T1DN. Moreover, these effects observed at cellular level may account for different renal protective effect of some gene modifier.

Cell signalling

SO024 FUNCTIONAL PROTEOMICS REVEALS AN IMPORTANT ROLE OF ER-CALCIUM BINDING PROTEINS IN ESTABLISHMENT OF CALCIUM HOMEOSTASIS UNDER OSMOTIC STRESS BY TALH-CELLS

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Introduction and Aims: Epithelial cells of the thick ascending limb of Henles loop (TALH) involve different mechanisms and pathways in their osmotic adaptation and stability. The alteration in the expression of ER stress proteins is a part of the TALH-cells reaction to osmotic stress (Dihazi et al. 2005, 2006).

Methods: In order to map the expression changes of ER stress proteins under high osmotic stress and to understand the role of this protein in osmotic stress resistance and in the establishment of calcium homeostasis, in gel electrophoresis approach with fluorescence labeled cysteines combined with mass spectrometry analysis were performed with established TALH model cell lines.

Results: The expression of ER stress proteins especially GRP78, GRP94, calreticulin, and Erp72, was significantly downregulated under osmotic stress ($p < 0.05$). GRP78 and calreticulin are actively engaged in regulation of free calcium concentration in the ER and the cytosol. Calcium imaging of NaCl stressed TALH-cells revealed time dependent elevation of Ca^{2+} ions concentrations in the cytosol arising from release out of endoplasmic reticulum stores. In parallel RT-PCR and Western blot analysis showed a progressive decrease in calreticulin and GRP78 expression. Thapsigargin treated TALH-cells exhibit no calreticulin or GRP78 downregulation under hypertonic stress when compared to control cells.

Conclusions: With our investigation we shed light on the important role of the down regulation of ER proteins in establishment of calcium homeostasis by TALH-cells under hypersomotic stress.

SO025 ★ INHIBITION OF mTOR ARREST HIGH GLUCOSE-INDUCING GLOMERULAR MESANGIAL CELLS SENESCENCE BY UPREGULATION OF SIRT1

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Introduction and Aims: The activation of mTOR plays a central role in diabetic nephropathy, but whether it is involved in senescence induced by high glucose in GMC still remain to be explored.SIRT1, an NAD-dependent deacetylase, is a principal modulator of pathways downstream of calorie restriction that produce beneficial effects on glucose homeostasis and insulin sensitivity.

Methods: In this study, the senescence of primary GMC was induced successfully by high glucose (30 mM), meanwhile, High glucose increased the expression of mTOR mRNA and protein as well as downregulated the level of SIRT1 protein. RNA interference and nicotinamide, a known inhibitor of SIRT1, were used to inhibit the expression of SIRT1gene and decrease the activation of SIRT1 that determine the contribution of SIRT1 to GMC senescence.

Results: The results showed RNA interference and nicotinamide can promote rat GMC senescence. NAD,which can activate SIRT1 by increasing the NAD/NADH ratio, while resveratrol, a polyphenolic SIRT1 activator, arrested senescence triggered by high glucose in rat GMC. Moreover, the mTOR pharmacological inhibitor rapamycin prevented senescence induced by high glucose in GMC by increaseing SIRT1protein expression and activity.

Conclusions: These results demonstrated for the first time that inhibition of mTOR can arrest the senescence of GMC induced high glucose, and its mechanism is increasement of SIRT1protein expression and activity.

SO026 APOCYNIN, INDEPENDENT OF SYSTEMIC HYPERTENSION, PREVENTS TUBULAR APOPTOSIS AND TUBULOINTERSTITIAL FIBROSIS IN TRANSGENIC MICE OVEREXPRESSING ANGIOTENSINOGEN

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Introduction and Aims: We have previously reported that transgenic (Tg) mice overexpressing the rat angiotensinogen (Agt) gene in their renal proximal tubular cells (RPTCs) develop hypertension, albuminuria and renal injury (Kidney Int 69:1016-1023, 2006). The present study investigated whether apocynin (an inhibitor of NADPH oxidase) interferes with the action of the intrarenal renin-angiotensin system (RAS), subsequently attenuating RPTC apoptosis and tubulointerstitial fibrosis, independent of systemic hypertension.

Methods: Adult male Agt-Tg mice were randomly divided into 4 groups: vehicle-treated Tg mice and Tg mice treated with apocynin, hydralazine (a vasodilator) or perindopril (an angiotensin-converting enzyme inhibitor). Non-Tg littermates served as controls. Systolic blood pressure (SBP) and albuminuria were monitored weekly until the animals were 20 weeks of age. Animals were then euthanized and their kidneys were processed immediately for assay of reactive oxygen species (ROS) [lucigenin assay], histology, and studies of apoptosis [terminal transferase-deoxyuridine triphosphate nick end-labeling or active caspase-3 and Bax immunostaining]. The mRNA and protein expression of pro-apoptotic genes [Bax and active caspase-3] and pro-fibrotic genes [transforming growth factor-beta 1 (TGF-β1), plasminogen activated inhibitor-1 (PAI-1) and collagen type IV] were quantified by real-time quantitative polymerase chain reaction assays and Western blotting, respectively.

Results: Agt-Tg mice displayed significantly elevated ROS production, hypertension (SBP: 117 vs 98 mm Hg, $p<0.001$), albuminuria (0.8 vs 0.3 μg albumin/mg creatinine, $p<0.01$), tubular apoptosis, and interstitial fibrosis as well as augmented Bax, active caspase-3, TGF-β1, PAI-1, and collagen type IV gene expression as compared to controls. Apocynin and perindopril markedly attenuated these changes, but apocynin had no effect on SBP. Hydralazine treatment prevented hypertension and tubulointerstitial fibrosis but not RPTC apoptosis. In vitro, apocynin and perindopril but not hydralazine prevented the increase of ROS generation, cellular apoptosis and pro-apoptotic gene expression in rat immortalized RPTCs overexperssing the Agt.

Conclusions: These data demonstrate that intrarenal RAS action on RPTC apoptosis and tubulointerstitial fibrosis is mediated, at least in part, via ROS generation and TGF-β1 signaling, independent of systemic hypertension (supported by the Kidney Foundation of Canada and the Canadian Institutes of Health Research).

SO027 ★ **RHOA AND RHOC DIFFERENTIALLY REGULATE THE EXPRESSION OF E-CADHERIN AND α-SMOOTH MUSCLE ACTIN DURING EPITHELIAL TO MESENCHYMAL TRANSITION**

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Introduction and Aims: The precise source of interstitial fibroblasts present in the fibrotic kidney is unclear but evidence suggests epithelial to mesenchymal transition (EMT) as a significant source. Recent evidence has demonstrated that the Rho family of monomeric GTPases are key modulators of EMT, but the individual roles of the Rho isoforms A, B, and C had not previously been investigated. In this study we focus on the adherens junction component E-cadherin, and the mesenchymal marker α-smooth muscle actin (α-SMA).

Methods: As an in vitro model of EMT we treated human kidney (epithelial) cells (HKC8) with 10ng/ml transforming growth factor β 1 and 10ng/ml epidermal growth factor for 5 days. 100nM short-interfering RNA (siRNA) was used to selectively silence RhoA, RhoB or RhoC. Two separate siRNA sequences were used to target each of the Rho isoforms in isolation and in combination. The efficiency and specificity of knock-down was ascertained by semi-quantitative PCR, quantitative PCR and immunoblotting. The expression of E-cadherin and α-SMA was determined by immunoblotting and semi-quantitative PCR. Rho-kinase 1/2 activity was blocked with 10μM Y27632. To study E-cadherin degradation 25μM proteasome inhibitor 1 (PI1) was utilised.

Results: siRNA-mediated knock-down of Rho mRNA, of at least 65%, was maintained for 5 days. Knockdown of mRNA for one isoform did not affect the expression of the others, and reduction of mRNA corresponded to reduction of protein abundance by 48 hours after transfection. EMT-induced upregulation of α-SMA protein was blocked by RhoA abrogation, but not RhoB or RhoC. This effect of RhoA is independent of Rho-kinase 1/2 activity, as Y27632 co-treatment did not rescue α-SMA expression. In our EMT model E-cadherin expression was abolished by 5 days. However, inhibiting RhoA or RhoC expression accelerated E-cadherin protein loss, which was complete by 48 hours. RhoA knock-down resulted in the loss of E-cadherin mRNA whereas knockdown of RhoC did not. Using PI1, the loss of E-cadherin caused by RhoC knock-down was rescued. PI1 did not, however, rescue E-cadherin loss induced by silencing RhoA.

Conclusions: The control of α-SMA gene transcription is specific to RhoA in our model, and is independent of Rho-kinase 1/2. Although the α-SMA promoter has previously been shown to be activated by TGFβ1 in a Rho-dependent, but Rho-kinase 1/2 independent, manner, this phenomenon was not known to be RhoA specific.

Pivotal to EMT is the loss of adherens junctions. Whilst Rho activity is known to be required for the maintenance of these junctions it has also been demonstrated that over-activity of Rho can lead to junction disassembly. Interestingly, we show that loss of RhoA appears to prevent expression of E-cadherin at the mRNA level whilst loss of RhoC does not. Conversely, inhibition of RhoC expression, but not that of RhoA, leads to increased degradation of E-cadherin. These results suggest that RhoA and RhoC are required to be expressed at a basal level to maintain E-cadherin at the adherens junction, but each does so by a different mechanism.

ERA-EDTA Fellows

SO028 **RAPID NORMALIZATION OF CAROTID INTIMA-MEDIA THICKNESS FOLLOWING RENAL TRANSPLANTATION IN NON-ATHEROSLCEROTIC PATIENTS WITH CHRONIC KIDNEY DISEASE CORRELATES WITH NORMALIZED SERUM URIC ACID LEVELS**

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Introduction and Aims: Chronic kidney disease (CKD) is associated with a dramatic increased risk of cardiovascular morbidity and mortality. In the general population, carotid intima media thickness (IMT) assessed using ultrasonography is an accepted marker of atherosclerotic plaque burden. However, as advanced CKD is also a state of marked fluid overload and endothelial dysfunction, we assessed the impact of these factors on IMT before and after renal transplantation.

Methods: We studied 406 patients with different stages of non-diabetic CKD (50% males, 46±12 years) and 58 kidney transplant recipients (57% males, 27±6 years), testing the relationship between IMT, assessed by ultrasonography, and selected biomarkers. We also investigated 80 healthy volunteers matched to the CKD group (50% males, 46±11 years) as well as 63 healthy volunteers matched for the transplant receipients group (48% males, 27±6 years), and these controls underwent similar testing.

Results: Patients had significantly elevated IMT compared to matched controls, and IMT correlated with eGFR. Following transplantation, IMT levels decreased concurrently with weight, and within 90 days post-transplantation had reached levels comparable to those of the controls, ie. 0.6 (range 0.4-0.7) mm. In multivariate analysis, eGFR ($p<0.001$), SBP ($p<0.001$), iPTH ($p<0.001$), HOMA ($p<0.05$) and DBP ($p<0.05$) were all found to be independently related to IMT levels in CKD patients. Similarly, only change in body weight ($p<0.05$) and in serum uric acid level ($p=0.001$) were independently related to the change of IMT level following transplantation.

Conclusions: While CKD was associated with a markedly increased IMT, following renal transplantation IMT was normalized within 90 days. This normalization was independently associated with a reduction of body weight (taken to represent normalization of fluid balance) and a decrease in serum uric acid levels. Our data thus suggests that in CKD patients, IMT is more closely associated with fluid status than with atherosclerosis.

SO029 **ABNORMAL CONTRACTILE ENDURANCE AND KINETICS TO ANG II IN RENAL RESISTANCE VESSELS OF NOTCH3 KNOCK OUT MICE**

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Introduction and Aims: Notch3 plays an important role in the differentiation and development of vascular smooth muscle cells. A mutation in this gene causes cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), the most common form of hereditary stroke disorder. Due to its clinical relevance, a large effort in recent years has been made to uncover the functions of this gene.

The kidney is of critical importance for pressure homeostasis. To study how Notch3 affects renal vascular function, basal morphology and microvascular responses to ANG II was investigated in the afferent arteriole (AA) obtained from mice lacking expression of Nothc3 (Notch3-/-).

Methods: Aas were isolated with the agarose infusion method (*Am J Physiol Renal Physiol, 291,* F140-7). Lumen area and length was traced in Olympus DP-Soft 5.0. Mean AA diameter was calculated using the formula (mean diameter) = (lumen area)/(lumen length).

Results: AA diameter was similar in WT (24.3±2.1 μm) and Notch3-/- (24.2±1.6 μm). Vessel wall thickness, however, was reduced in Notch3-/- (14.5±1.0 μm) compared to WT (19.9±1.7 μm, $p < 0.01$). Further analysis revealed that cell wall diameter measured at opposite positions in randomly chosen cross-sections of the vessels varied more in Notch3-/- (Δ9.7±1.5 μm) than in WT (Δ2.9±0.4 μm, $p < 0.001$), indicating an asymmetric vessel wall structure of the Notch3-/- AA.
During the initial 5 s after ANG II stimulation (10^{-7} M), AAs from Notch3-/- animals displayed an exaggerated lumen diameter response (67±5% of baseline) compared to WT (86±5% of baseline, $p < 0.05$). In contrast, the sustained contractile response measured at 180 s was much smaller in KO compared to WT (83±5% of baseline vs. 61±6% of baseline, respectively, $p < 0.05$). Similar differences were found at 10^{-8} M ANG II.
To test dependence of extracellular Ca^{2+}, the lumen diameter response was studied in calcium free medium (EGTA 10^{-4} M). The exaggerated initial response to ANG II 10^{-7} M in Notch3-/- vessels was maintained, demonstrating importance of intracellular Ca^{2+} release. Furthermore, the sustained contractile response was unaltered in KO vessels after Ca^{2+} removal (83±5% of baseline vs. 89±8% of baseline with and without Ca^{2+}, respectively, at 180 s), whilst WT contractility was reduced (61±6% of baseline vs. 85±8% of baseline with and without Ca^{2+}, respectively, at 180 s, $p<0.05$).
Conclusions: The present data suggests that renal resistance vessels of Notch3-/- display an abnormal defective response to vasoconstrictors. This defect is probably due to a structural deficiency; a thinner and asymmetrically organized vessel wall. As a likely consequence, compensatory $Ca^{2+}{}_i$ mobilization from intracellular stores may occur during the initial phase of contraction after ANG II stimulation, resulting in an exaggerated early response that AAs of the Notch3-/- are unable to sustain. This result indicates that a functional Notch3 gene plays an important role in the control of renal vascular resistance.

SO030 CD36 27645 ins/del POLYMORPHISM IS ASSOCIATED WITH CARDIOVASCULAR MORTALITY IN END-STAGE RENAL DISEASE PATIENTS

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Introduction and Aims: CD36 is an 88kDa glycoprotein which is present on the cell surface of many different cell types including monocytes/macrophages, endothelial cells, and myocytes. CD36 has numerous functions such as being a scavenger receptor, long chain fatty acid transporter, and receptor for thrombospondin-1. In CD36-deficient patients, increased susceptibility to type 2 diabetes has previously been documented, while CD36-KO mice have been shown to have retarded atherosclerosis progression. The aim of the study was to evaluate the association between a common CD36 polymorphism (CD36 27645 ins/del) and comorbidities as well as survival in end-stage renal disease (ESRD) patients.
Methods: CD36 27645 ins/del polymorphism was evaluated in 310 stage 5 CKD patients, close to start of renal replacement therapy, by size separation through gel electrophoresis. For polymorphism frequency comparison, an additional cohort of 562 healthy controls was studied. Fasting blood samples were analyzed at baseline for routine biochemistry and hematology, markers of inflammation and oxidative stress (8-hydroxydeoxyguanosine; *8-OHdG*) and cardiac injury (troponin T). The patients were followed prospectively, and the three-year all cause and cardiovascular mortality was analysed.
Results: The distribution of the CD36 27645 ins/del polymorphism was similar in ESRD patients and in the control group. The CD36 27645 *del* variant was associated with lower concentration of hemoglobin, and higher levels of troponin T and 8-OHdG in an allele-dependent manner, as well as with higher prevalence of diabetes mellitus. Furthermore, the CD36 27645 *del* variant was an independent predictor of three-year cardiovascular mortality in ESRD patients (HR 2.56; $p<0.002$), following adjustment for: age, gender, diabetes mellitus, cardiovascular disease and inflammation.
Conclusions: CD36 27645 ins/del polymorphism is associated with cardiovascular mortality in ESRD patients. Since the presence of the CD36 27645 *del* variant was associated with diabetes, anemia, as well as with signs of cardiac injury and oxidative stress in this patient population, its apparent impact on cardiovascular mortality could be due to proatherogenic effects, conceivably mediated via an altered function of CD36.
Disclosure: Bengt Lindholm is employed by Baxter Healthcare.

SO031 POLYCYSTIN-2 IS INVOLVED IN THE CONTROL OF CENTROSOME DUPLICATION

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Introduction and Aims: Polycystin-2 (PC-2), a protein involved in autosomal dominant polycystic kidney disease (ADPKD) encoded by *PKD2*, is a non-selective cation channel, recently implicated in the function of the primary cilia. PC-2 has a control on the intra-cellular calcium level. The basal body is a modified centrosome. The function of *PKD2* in the centrosome biology is unknow. An abnormal centrosome number is frequently observed in cancer cells and linked to genomic instability. Our aim is to study the impact of modulation of PC-2 in vivo on the centrosome control number.
Methods: We used two transgenic mice lineage. One overexpressed the human polycystin 2, the second is a knock out mouse for *Pkd2*. We extract fibroblasts from transgenic mice and wild type mouse at various ages. We also knock down *Pkd2* expression in wild type fibroblast using siRNA. Genomic stability was studied by FISH. The centrosome number was counted after labelling with an antibody against γtubulin. The activity of the calcium/calmodulin-dependent protein kinase II (CaMKII) was inhibited with the KN-93 inhibitor.
Results: We observed that fibroblasts from a transgenic mouse line overexpressing human *PKD2*, present with mitotic instability. The karyotype analysis schowed abnormal number of chromoses. This mitotic instability is linked to the formation of supernumerary centrosome (30% of fibroblasts had more than 2 centrosomes). Knocking down *Pkd2* expression in normal mouse fibroblasts by siRNA results in an abnormal over-duplication of centrosomes. We also observed centrosome overduplication in mouse embryonic fibroblasts derived from homozygous mutant $Pkd2^{LacZ/LacZ}$ KO embryos. In the fibroblast derived from heterozygotes, the abnormal centrosome number is intermediate betwen KO and wild type. This phenotype is corrected by incubation of transgenic or KO cells with an inhibitor of calcium/calmodulin-dependent protein kinase II (CaMKII), indicating an implication of this pathway in centrosome overduplication induced by perturbation of PC-2 expression. The wild type fibroblast, as previously described, exhibited an abnormal centrosome number after incubation with KN-93. We propose that PC-2 abnormal expression protects normal cells from centrosome overduplication after CamKII inhibition. In addition in the fibroblasts with an excessive number of centrosome we observed no primary cilia or more than one primary cilia.
Conclusions: Pc-2 plays a role in the control of cell division through the control of centrosome duplication. The anomalies in the number of centrosome is linked to genomic instability in cancer via the perturbation of mitosis. We propose a model where perturbation of *PKD2* expression generates excessive centrosome duplications. The abnormal number of centrosome could be responsive for the defective mitotic axis observed in tubules of polycystic mice. In addition we observed that the overduplication of centrosome secondary of CamKII inhibition could be inhibited by non regulated PC-2 expression. This could be a new way to control centrosome number in cancerous cells.

SO032 ACUTE METABOLIC ACIDOSIS PROMOTED BY THE CALCINEURIN INHIBITOR FK506 (TACROLIMUS) IN RAT KIDNEY

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Introduction and Aims: Calcineurin inhibitors like FK506 (Tacrolimus) are routinely used for immunosuppression following transplantation in conjunction with an antiproliferative agent. The use of these drugs is limited by

many side effects such as reduction of the glomerular filtration rate and renal tubular acidosis (RTA), mainly of the distal type. Therefore, we investigated the effect of FK506 on rat kidney function and expression of key acid-base transporters to elucidate the underlying molecular mechanisms/changes.
Methods: We used a rat model injecting the animals subcutaneously with 1mg/kg body weight FK 506 for 11 or 16 days. Control animals were injected with 0.9% NaCl solution for the same time period. In addition, metabolic acidosis was induced by adding 0.28M NH_4Cl to drinking water for 2 or 7 days.
Results: Under normal conditions systemic acid base status was similar in both groups. However, after 2 and 7 days acid loading with 0.28M NH_4Cl, the Tacrolimus treated animals showed a more severe metabolic acidosis, elevated blood glucose levels, and reduced GFR. Excretion of phosphate was lower in the Tacrolimus treated animals with an acid load. Despite the more pronounced acidosis in the Tacrolimus group after 2 days acid loading, urine pH was more alkaline indicating distal RTA. Interestingly, ammonium excretion was significantly higher in these animals. After 7 days acid load all differences related to acid-base handling were equalized in both groups. Western blot analysis revealed reduced expression of the a4 and B1 subunits of the vacuolar H^+-ATPase under normal conditions but a higher abundance of a4 after induction of metabolic acidosis in cortex and medulla. The expression of the acid-secretory type A intercalated cell specific $Cl^-/HCO3^-$ exchanger AE1 in total kidney was lower under normal conditions and significantly increased after induction of metabolic acidosis for 2 days. The abundance of the type IIa and IIc Na^+/phosphate cotransporter as well as the B2 subunit of the vacuolar H^+-ATPase were reduced under normal conditions in the Tacrolimus treated animals and significantly increased after acid loading.
Conclusions: In summary, treatment with Tacrolimus did not induce renal tubular acidosis in rats under baseline conditions. Further, Tacrolimus treated rats developed a more severe hyperchloremic metabolic acidosis under an acute oral acid load after 2 days, but this effect was equalized after 7days, indicating an acute and transitory impairment of the renal adaptation to acidosis. Key transport proteins involved in renal acid base handling as vacuolar H^+-ATPase, the $Cl^-/HCO3^-$ exchanger pendrin or the acid-secretory type A intercalated cell specific $Cl^-/HCO3^-$ exchanger AE1 were downregulated in animals treated with Tacrolimus. However, induction of metabolic acidosis contributed to an appropriate upregulation of these transporters under acid challenge.
In conclusion, treatment with Tacrolimus induced transient renal tubular acidosis after an oral acid challenge of 2 days in rat. Thus, Tacrolimus may have a decelerating effect on the renal adaptation to an acute acid challenge.

SO033 GLUCOSE DEGRADATION PRODUCTS MAY IMPAIR THE HEAT SHOCK RESPONSE

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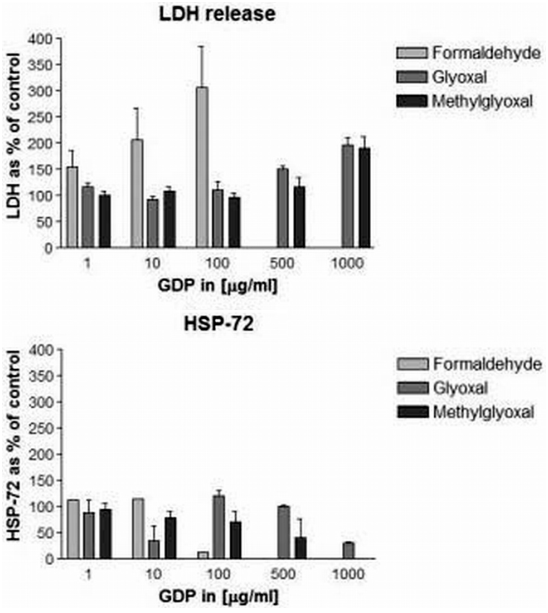
Introduction and Aims: Cellular damage is balanced between toxicity induced injury and counteracting cellular responses. We have previously shown that exposure of mesothelial cells to peritoneal dialysis fluids (PDF) not only caused toxic injury but also induced cytoprotective heat shock proteins (HSP). Recently glucose degradation products (GDPs) were identified as probably the most important biocompatible impairing component of PDF. But up to now the role of GDPs on HSP expression is not known. This study was performed in order to evaluate the impact of GDPs on the heat shock response.
Methods: Mesothelial Cells (Met5a) where incubated in the presence of increasing doses of eight glucose degradation products found in PDF (Acetaldehyde, Formaldehyde, Glyoxal, Methylglyoxal, Furaldehyde, 5-HMF, 3-DG and 3.4-DGE). After 24 hours of exposure, viability was assessed by LDH release (as marker for toxic injury) and protein was harvested for HSP-72 determination by Western blotting (as marker for cytoprotective cellular response). All data presented as % of control ± SEM. Formaldehyde (n=7), Glyoxal (n=3), Methylglyoxal (n=5) for LDH release and Formaldehyde (n=1), Glyoxal (n=3), Methylglyoxal (n=5) for HSP-72.
Results: From the eight GDPs tested three (Formaldehyde, Glyoxal and Methylglyoxal) resulted in a dose dependent increase of LDH release (1st figure). Interestingly, these increases were associated with absence of adequate HSP induction (2nd figure). This is in contrast to results obtained from past studies regarding other biocompatibility impairing components of PDF like acidic pH or lactate which resulted in significant HSP induction before cellular viability was impaired. The other GDPs did not result in significant LDH release or HSP-72 expression.



Conclusions: Inadequate HSP induction by cellular stressors has the potential to deteriorate cellular oucome following exposure to cytotoxic PDF. GDPs might thus introduce a novel way of adding to PDF bioincompatibilty by impairing the counteracting cellular stress responses.
Disclosure: This abstract was possible thanks to a long-term fellowship given by ERA-EDTA.

Chronic kidney disease

SO034 ★ THE INFLUENCE OF CHRONIC KIDNEY DISEASE ON HIP-FRACTURE RELATED MORTALITY IN OLDER PEOPLE IN THE UNITED KINGDOM

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Introduction and Aims: Severe chronic kidney disease (CKD) is associated with mineral disorders which can result in pathological bone structure, and increased risk of falling from associated comorbidity and therapy. Clinical significance is unclear especially in older people who have also increased prevalence of osteoporosis with incident hip fractures. The aim of this study was to examine whether CKD at older ages is associated with hip fracture related mortality.
Methods: Cohort study of people aged 75 and over participating in a cluster randomised trial of health and social assessment of older people in the community between 1994-8 in the UK. There were 13177 (87%) participants in 53 general practices who had a serum creatinine measured in local laboratories at baseline. Estimated glomerular filtration rate (eGFR) was derived from the Modification of Diet in Renal Disease formula (MDRD). Analyses are based on deaths up to end November 2005 for any reported fracturing of the hip based on ICD codes on the death certificate sent to the Office for National Statistics. Participants were also

followed up in detail over a two year period after inclusion into the trial to produce a record of all hospital admissions and corresponding causes. We used propensity scores to appropriately adjust for all potential confounders (relating to both falls risk and bone structure) in the Cox regression models.
Results: There were 124 hospital admissions for hip fractures in the first two year, and 84 hip fracture related deaths over 84117 person years of follow-up. In analyses with outcome hip fracture related deaths compared to an eGFR>60 ml/min/1.73m^2, the age and sex-adjusted hazard ratios for eGFR 45-59 and <45 ml/min/1.73m^2 were 1.06 (95% confidence interval: 0.71, 1.58), and 1.97 (1.12,3.50), respectively. When adjusting for all confounders using propensity scores, the adjusted hazard ratio for participants with eGFR<45 ml/min/1.73m^2 compared to those with eGFR above was 1.81 (1.11, 2.94). There was no evidence for an association between eGFR and hospital admissions for hip fracture in the first two years after trial inclusion.
Conclusions: Amongst older people, an eGFR of <45ml/min/1.73m^2 is independently associated with a two fold increase in hip fracture related mortality. Larger studies with longer follow up are needed to examine the association of CKD with incident hip fractures, and thereby separate potential effects of CKD on fracture risk and case fatality.

SO035 ★ INFLUENCE OF DIET QUALITY AND DIET PATTERNS ON ALBUMINURIA IN WOMEN

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Introduction and Aims: Microalbuminuria (MA) is a strong independent predictor of CVD events and all-cause mortality. Sparse data are currently available, however, on how potentially modifiable risk factors such as diet may influence albuminuria.
Methods: We identified 3121 women participating in the Nurses' Health Study, an established longitudinal cohort study, who had diet data and urinary albumin-to-creatinine ratios (ACR) measured on urine collected in the year 2000. This group included 674 women who comprised a sub-study of type 2 diabetics. Cumulative averaged diet quality scores and diet patterns (prudent vs. Western) over 14 years were derived from validated semi-quantitative food frequency questionnaires from 1984, 1986, 1990, 1994, and 1998. Higher diet quality scores generally reflect greater intake of vegetables, fruits, and whole grains and lower intake of red and processed meats and saturated fats.
Multivariable (MV) logistic regression was used to assess for associations between quartiles of energy-adjusted diet quality indices, including the Healthy Eating Index (HEI), Revised Healthy Eating Index (RHEI), Diet Quality Index Revised (DQIR), Recommended Food Score (RFS), and Mediterranean Diet Index (MED), and presence of MA or greater (defined as ACR >= 25 mcg/mg).
Results: Mean age was 66±6.7 years, 89% Caucasian, 54% with HTN, 23% with diabetes, 5% with CVD, 6% current smokers, mean BMI 27.1±5.8 kg/m^2, and mean eGFR 77±18 ml/min/1.73 m^2 by 4-variable MDRD equation at time of ACR measurement. In this group, 177 (5.6%) women had MA or greater. MV models revealed that higher quartiles of diet quality scores were consistently associated with decreased odds ratio of presence of MA or greater with the exception of RFS (Table 1). Furthermore, the highest quartile of Western diet pattern score was significantly and directly associated with an OR 2.22 (95% CI 1.20 to 4.10, p-for-trend=0.007) for MA or greater.

Table 1. Multivariable* Odds Ratios and 95% CI for Diet Quality Scores by Quartile and Presence of MA or Greater

	Quartile 1	Quartile 2	Quartile 3	Quartile 4
HEI	1.0 (referent)	0.65 [0.41, 1.04]	0.68 [0.43, 1.08]	0.62 [0.38, 1.03]
RHEI	1.0 (referent)	0.63 [0.40, 0.98]	0.69 [0.45, 1.07]	0.62 [0.38, 1.01]
DQIR	1.0 (referent)	0.55 [0.34, 0.88]	0.59 [0.37, 0.94]	0.67 [0.42, 1.07]
RFS	1.0 (referent)	0.80 [0.50, 1.28]	0.84 [0.52, 1.36]	0.99 [0.60, 1.64]
MED	1.0 (referent)	0.63 [0.40, 0.98]	0.70 [0.45, 1.08]	0.57 [0.35, 0.93]

*All MV models adjusted for age, caloric intake, HTN, BMI (kg/m^2), physical activity (METS/week), diabetes, cardiovascular disease, and ACE-inhibitor/ARB medication use.

Conclusions: Higher diet quality is inversely associated with presence of MA whereas the Western diet pattern high in red meat, refined grains, sweets, and high-fat dairy products is directly associated with presence of MA. Therefore diets higher in fruits, vegetables, whole grains, and fish may protect against development of MA and deserve further study.

SO036 ★ PROGNOSTIC ASSOCIATION OF SERUM PHOSPHATE IN PATIENTS WITH CHRONIC KIDNEY DISEASE NOT ON DIALYSIS

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Introduction and Aims: Serum phosphate has been related to significant morbidity and mortality in the dialysis population. Less is known about the relationship between serum phosphate levels and mortality in patients with chronic kidney disease (CKD) not on dialysis although one study has shown an association between hyperphosphataemia and mortality in a veteran cohort with raised creatinine measurements. We investigated the prognostic associations of baseline serum phosphate in the chronic renal insufficiency standards implementation study (CRISIS) which is a prospective epidemiological study of patients with CKD stages 3-5 not on dialysis.
Methods: 934 patients were available for study and their mean follow up period was 1260 days (± 539). Serum phosphate was divided into quartiles in order to evaluate associated significant factors. Linear regression was also performed and adjusted for eGFR, PTH, proteinuria, diabetes, cardiovascular disease and age.
Results: The mean age of the patients was 64.5 (± 14.2) years, estimated glomerular filtration rate (eGFR) 31.1ml/min (± 14.5), 34% female, serum phosphate 1.2mmol/l (±0.3), corrected calcium 2.3mmol/l (±0.2), parathyroid hormone 90.2pg/ml (±96.3), proteinuria 1.1g/d (±1.8) and 30.4% had cardiovascular disease. Increasing phosphate was positively associated with mortality ($p<0.005$) with an absolute risk reduction of 14.3% and a relative risk reduction of 21.2% demonstrable between the lowest (phosphate <1mmol/l) and highest (phosphate >1.35mmol/l) quartiles. In total there were 244 (26%) deaths during the period of follow up. Serum phosphate was also positively associated with parathyroid hormone, proteinuria, diabetes, smoking and female sex. It was negatively associated with haemoglobin, diastolic blood pressure and weight. No relationship was found with baseline eGFR. On multivariate linear regression a significant association was maintained with mortality, parathyroid hormone, proteinuria, haemoglobin, gender, diabetes, weight and smoking.
Conclusions: This prospective study shows that serum phosphate is independently associated with all-cause mortality in chronic kidney disease stages 3-5 (not on dialysis). This effect holds true even with serum phosphate values within the accepted normal range - eg 87% of patients had a serum phosphate less than 1.5mmol/l (KDOQI upper limit in CKD). Clearly phosphate control is of major importance in CKD; further studies are merited in order to investigate whether lower targets for phosphate control are indicated in CKD patients.
Disclosure: Funded by an educational grant from Genzyme.

SO037 FLUID OVERLOAD AND MALNUTRITION ASSESSED WITH BIOIMPEDANCE SPECTROSCOPY (BIS) ARE STRONG PREDICTORS OF MORTALITY IN HEMODIALYSIS PATIENTS

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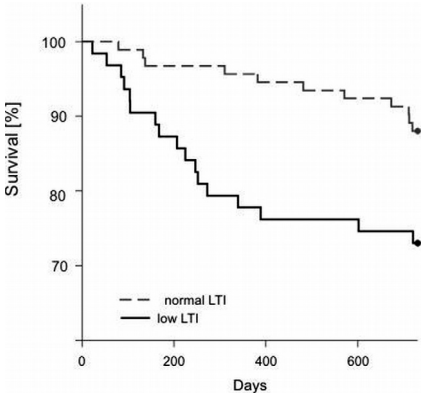
Introduction and Aims: Malnutrition and fluid overload (FO) are common problems in hemodialysis (HD) patients. Traditional bodycomposition assessment methods (e.g. bioimpedance analysis (BIA) and Dual X-Ray Absorptiometry (DEXA)) cannot separate fluid overload from muscle mass. Thus an objective and quantitative assessment of hydration- and nutrition status is currently not possible. A novel high-frequency bioimpedance-spectroscopy device (BCM-Body Composition Monitor, FMC) determines fluid overload and Lean Tissue Index (LTI) based on a novel body

composition model (AJCN 85, 2007). In previous studies it was shown that the device can be used to assess FO (EDTA 2007) and malnutrition. The aim of this study was to show if protein malnutrition or fluid overload is the major predictor of mortality in HD patients.
Methods: LTI and FO were determined via the BCM in 156 patients. To evaluate LTI and fluid overload, reference ranges were set up on basis of a reference population (RP) of n=1000 normal subjects between 18–85 years. To analyse protein malnutrition patients were separated into LTI groups (*low* if LTI < 10th percentile of the RP, *normal* if LTI was > 10th percentile of the RP). Patients were regarded as fluid overloaded, when FO was > 2L at the start of the HD-treatment. Survival analysis was performed using the Gehan-Breslow method, adjusting for age, sex and diabetes. The relative risks were calculated on the basis of the odds ratios.
Results: Mean time on HD before measurement was 3.5±5.7 years, mean age was 67±13 yrs. After 2 yrs, 18% of all patients had died. The *low* LTI group showed a significantly greater mortality (p=0.009) compared to the normal LTI group. Also the *high* FO group showed a significantly increased mortality (p=0.005). After 2 yrs, 27% of patients in the low LTI group had died, while only 12% had died in the normal group. The subgroup of patients with LTI<10% and FO>2L had a 2,4 fold increased risk of dying compared to the subgroup with normal LTI and normal FO.

Relative Risk in subgroups

group	% of patients	RR
All patients in study	100	1
Low LTI (LTI<10%)	47	1,3
Normal LTI (LTI>10%)	53	0,7
Normal FO (FO<2 L)	64	0,88
High FO (FO>2L)	36	1,22
Low LTI & high FO (LTI<10%, FO>2,0 L)	25	1,53
Normal LTI & low FO (LTI>10%, FO<2,0 L)	30	0,63



Conclusions: Malnutrition and fluid overload are both influencing the mortality risk of HD patients. LTI and FO in combination with the reference ranges are key indicators for survival and can be easily obtained in all dialysis patients using the BCM-Body Composition Monitor.
Disclosure: Some of the authors are employees of Fresenius Medical Care.

SO038 CORONARY FLOW RESERVE IS UNAFFECTED BY CHRONIC KIDNEY DISEASE IN PATIENTS WITHOUT SYMPTOMATIC CORONARY ARTERY DISEASE

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Introduction and Aims: Among patients with end-stage renal disease (ESRD) the risk of cardiovascular disease is 10 to 20 times higher than in general population. Even minor renal dysfunction is a powerful cardiovascular risk factor. Numerous heterogeneous cardiovascular abnormalities have been described in patients with renal dysfunction. One final common pathway seems to be endothelial dysfunction.
The purpose of this study was to investigate whether decreasing glomerular filtration rate (GFR) is associated with functional abnormalities in coronary flow or in peripheral vasculature in patients with chronic kidney disease (CKD) but without symptomatic coronary artery disease.
Methods: Myocardial blood flow was measured at baseline and during dipyridamole-induced hyperaemia (intravenously 0.56 mg/kg body weight in 4 min) by means of positron emission tomography (PET) and oxygen-15-labeled water. The peripheral artery endothelial function was examined by measuring flow-mediated dilatation using ultrasound at rest and during reactive hyperaemia in the brachial artery. Patient demographics are shown in table. Baseline characteristics were similar between the groups, with the exception of use of hypertensive medication by CKD patients, all but one used angiotensin enzyme inhibitor or angiotension receptor blocker. The amount of proteinuria was approximately 0.5 g/day in CKD patients. Diabetic patients were excluded.
Results: Baseline myocardial blood flow was significantly higher in CKD (stage 3-5) patients than in healthy controls (p<0.001). The baseline flow was equal in all CKD stages. A singnificant and equal increase in myocardial blood flow was detected in every group after dipyridamole infusion thus leading to equal coronary flow reserve in every group (table).
The peak flow mediated dilatation response (peak FMD %) was highest in healthy controls, 9.6±2.5%. It was significantly lower in CKD patients but didn't differ according to CKD stage (groups 3 to 5: 6.0±5.0% vs. 5.7±4.6% vs. 5.6±3.2%, p<0.03.)

Group	N	Age	eGFR ml/min	MAP basal mmHg	Baseline flow ml/min/g	Stimulated flow ml/min/g
Controls	10	59±8	76±5	100.8±7.5	0.873±0.139	2.471±0.850
CKD 3	5	54±10	37±7	97.1±3.7	1.175±0.182*	3.304±1.280
CKD 4	12	54±10	21±5	102.6±6.9	1.247±0.324*	3.172±1.214
CKD 5	6	54±5	12±2	94.4±9.5	1.226±0.193*	2.606±1.038

*p<0.001 controls vs. CKD3-5.

Conclusions: To our knowledge this is the first report of myocardial blood flow measurements using PET techniques in CKD patients. Interestingly our results show that baseline myocardial blood flow is increased in CKD patients compared to healthy controls. The coronary flow reserve is also preserved despite of declining eGFR. However, CKD patients showed impaired endothelial function peripherally. The mecanisms regulating vascular responses in the heart and peripheral vasculature may be different.

Epidemiology 1

SO039 MORTALITY RATE IN INCIDENT DIALYSIS PATIENTS IN THE LAST TWO DECADES

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Introduction and Aims: Mortality rate of patients receiving Renal Replacement Therapies (RRTs) has remained stable in the last decades. We hypothesized that the adjusted survival probabilities may have improved over time as new patients are getting older and sicker. The effect of dialysis start period on mortality rates was studied in a large cohort of incident RRT patients in Lombardy (northern Italy).
Methods: Data were obtained from the database of the RLDT registry. From 1986 to 2005, 11.916 patients (age 63±15) were admitted to RRT programs in 32 dialysis Units in Lombardy. The effect of the period of dialysis start on survival was analyzed using Cox proportional hazards regression, taking into account age, gender, race, diabetes, comorbids, dialysis modality and center of treatment and their first order interaction terms. Patients were censored at the 4th yr of follow up, at 31th december 2005 or when transplanted or transferred to a Dialysis Center outside of Lombardy.
Results: From 1986 to 2005 the mean age of patients starting RRT increased from 58 to 65 yrs. The annual admittance rate increased from 114 to 172 pmp. During follow up 6236 pts died, 47% due to cardiovascular causes. Annual death rate remained stable (12.3% in 1986, 13% in 2005). The relative hazard for death was 14% lower in the period 1986-2005 as compared to the previous decade (RR 0.86; 95% CI: 0.77, 0.95). This period

effect was independent of age, gender, presence of diabetes, neoplasia and cardiovascular diseases at dialysis commencement. In addition the period effect was not modified by level of considered covariates.
Conclusions: Once factors impacting outcomes are taken into account, patients who enter RRT programmes today appear to survive longer compared to the past. This suggests an improvement of the patient care in the last yrs. The potential impact of specific technological advances and new pharmacological therapies deserve further large scale investigations.

SO040 DYNAMICS OF SYSTOLIC BLOOD PRESSURE PREDICTS MORTALITY IN INCIDENT HEMODIALYSIS PATIENTS – APPLICATION OF A MARKOV MODEL

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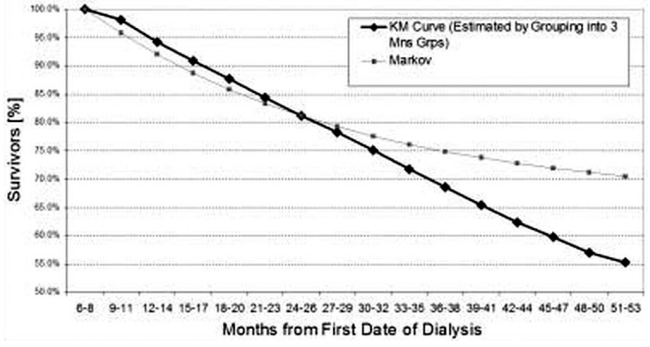
Introduction and Aims: Pre-dialysis systolic blood pressure (SBP) below 120 mmHg is associated with poor survival in chronic hemodialysis (HD) patients. This study aimed to test the hypothesis that in addition to low SBP the change of SBP is a predictor of mortality.
Methods: We studied all incident Renal Research Institute and New York Dialysis Services (RRI and NYDS) in-center maintenance hemodialysis patients with their first dialysis date (FDD) between 10/1/2002 and 12/31/2006. Pre-dialysis sitting SBP (pre-sSBP) was collected for every in-center treatment and averaged for each month of the study period. Only the patients who were alive for at least 240 days from FDD were included in the analysis. A Markov model with two absorbing states (death; censoring for reasons outlined below) was developed based on the median pre-sSBP of months 6 to 8 from FDD (initial state matrix). Patients were divided into three groups of pre-sSBP, <120, 120-160, and >160 mmHg. Survival status was recorded in each patient, and the Markov transition matrix was computed based on the outcomes during months 9 to 11 from FDD. Patients were censored for transfer to another unit, kidney transplant, discharge from the dialysis center for other reasons, or end of study period. For comparison with the Markov model, a Kaplan Meier (KM) survival curve was constructed for the same cohort.
Results: We studied 4,494 incident HD patients (55% females, mean age ± SD 63.8±15.5 years, 49% with diabetes mellitus). The group allocation in the initial state matrix was 6.4% (<120 mmHg), 62.7% (120-160), and 30.9% (> 160 mmHg). The transition matrix is shown in Table 1.

Table 1: Transition matrix. The elements of the matrix describe the probability of being in a particular group.

		<120	120-160	>160	Died	Censored
Current Stage	<120	0.52	0.27	0.01	0.13	0.08
	120-160	0.04	0.71	0.12	0.04	0.09
	>160	0.00	0.26	0.64	0.03	0.07
	Died	0.00	0.00	0.00	1.00	0.00
	Censored	0.00	0.00	0.00	0.00	1.00

Up to 30 months, the Markov model accurately predicted survival (Fig. 1).



Conclusions: Survival in incident HD patients can be accurately predicted for up to $2\frac{1}{2}$ years by means of a Markov model based on pre-sSBP. We hypothesize that a low and/or falling pre-sSBP may represent the common terminal pathway or different pathological processes, such as cardio-vascular disease, chronic inflammation, infection, and poor nutrition. Since the pattern changes after $2\frac{1}{2}$ years, it may be necessary to apply time-dependent transition matrices. In addition, Markov models stratified by race, gender, age, and diabetes mellitus status may prove to be insightful. Further evaluation of this concept could result in a useful indicator of mortality.

SO041 TREND ANALYSIS OF THE INCIDENT ESRD PATIENTS IN FLANDERS [BELGIUM] FROM 2000 TILL 2006

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Introduction and Aims: Renal registries offer the possibility to closely monitor the renal population and their characteristics, and to anticipate the need for renal services. We analyzed the data of the regional registry of the Dutch speaking nephrologists society of Belgium (NBVN) to study the trends of demography and kidney disease in incident patients with end-stage renal disease (ESRD).
Methods: The NBVN registers all ESRD patients within the Flanders region, using an on-line database. Mandatory criteria are: patient age, gender, kidney disease, body weight, treatment modality (hemodialysis, peritoneal dialysis, kidney transplantation) at the start of ESRD; date of change of ESRD treatment; and date and cause of death. An epidemiologist monitors and checks the data, informs centres on incomplete or illogical data, and does also on-site audits. For this study, we extracted all incident ESRD patients between Jan 1, 2000 and Dec 31, 2006.
Results: The number of incident ESRD patients is rising each year, from 882 in 2000 to 1152 in 2006. The relative annual increase, however, is levelling off from almost 10% before 2003 to only 3% after 2003.
Age category: In the population younger than 65 yrs, the incidence of ESRD did not change over the study period (N= 350±10). In the category 65-74 years, the incidence rose from 280 in 2000 to 338 in 2004, and decreased afterwards: 327 in 2005 and 308 in 2006. The group 75 - 84 yrs showed a constant increase from 220 in 2000 to 424 in 2006. The elderly group over 85 yrs old initially grow till 2003, had a major drop in 2004, followed by an increase in 2005 and 2006. Corrected per 105 population, the lowest ESRD incidence is observed in the <65 yrs age group [7/105 population], being constant over the 7 years. The mean annual incidence of patients in the 65-74 yrs group is 54 per 105 population, with some decrease during the last two years. The highest incidence of ESRD per 105 population is present in the 75-84 yrs group, and steadily increases over the study period. The annual increase of the >85 yrs group per 105 population was abruptly ceased in the 2004 and 2005, but recovered in 2006.
Origin of kidney disease: As of 2005 there is an overall slowing increase of new diabetic ESRD patients. This drop is particularly seen in the 65-74 yrs age group. The other age groups show either an increasing incidence (75-84 yrs) or a stable inflow (<65 yrs, and 85+ yrs). The incidence of ESRD caused by vascular kidney disease annually grows by 5%.
Conclusions: Trend analysis of the incident ESRD patients in Flanders region show a marked decrease of new diabetic ESRD patients and an levelling off of the 65-74 yrs age group; this might be the effect of the diabetic and renal care guidelines, implemented as of 2000. The low influx of >85 yrs in 2004-2005 is probably explained by "natural selection" of the heat wave in 2003.

SO042 COMPARISON OF SURVIVAL IN PERITONEAL DIALYSIS AND HEMODIALYSIS ITALIAN ESRD PATIENTS: A PROPENSITY SCORE ANALYSIS

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Introduction and Aims: ESRD patients addressed to peritoneal dialysis (PD) are likely in a better health status than the hemodialysis (HD) ones, thus the differences in survival between the two modalities may be hardly biased by selection.To compare the survival between PD and HD patients, the historical cohort of subjects starting renal replacement therapy in 8

Italian regions enrolled in the Italian Dialysis and Transplantation Registry between 2000 and 2005 was followed up to 31/12/2005.
Methods: First modality choice was considered on an intention-to-treat basis. The Kaplan-Meier survival functions for PD and HD were compared. The propensity score for PD or HD patients was estimated with logistic regression using year of incidence, sex, age, primary kidney disease, known comorbidities (diabetes, ischemic heart disease, heart failure, hypertension, liver disease, stroke, peripheral vascular disease, cancer and pulmonary disease), and region as predictive variables. Patients were classified in blocks balanced according to the propensity score. The survival analysis was repeated stratifying over propensity score blocks.
Results: 18841 subjects started RRT in the considered period: 2313 on PD and 16528 on HD. In 5 years overall mortality was lower in PD than in HD (log-rank test: χ^2 = 11.74, p=0.0006), and in the first year PD patients had a significant survival advantage (RR=0.65, 95%CI: 0.57 – 0.74). After propensity score stratification, no difference in survival between the two treatments was observed (log-rank test: χ^2=0.72, p=ns), but the survival advantage in the first year for PD was yet present (RR=0.71, 95%CI: 0.62 – 0.81), while a survival disadvantage was observed in the second and in the third year (RR=1.18, 95%CI: 1.00 – 1.39 and RR=1.33, 95%CI: 1.09 – 1.61 respectively).
Conclusions: Our nation-wide findings confirm observational studies performed in other countries. Propensity score adjustment accounts only for observed covariates, thus we may state that, conditioning on age and health status, PD and HD promise the same overall survival. This result emphasizes the selection bias in the choice of renal replacement modality. Other unknown confounders, such as renal residual function, and bias, particularly lead time bias, may account for the differences observed at specified analysis times.

SO043 INTIMA AND MEDIA THICKENING, CALCIFICATION AND INFLAMMATION IN PATIENTS WITH EARLY AND LATE STAGES OF RENAL FAILURE

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Introduction and Aims: Death from cardiovascular causes is the major cause of death in patients with endstage renal failure. While patients with renal failure have a high prevalence of classical coronary risk factors, there is increasing evidence that atherosclerosis, i.e. the evolution and composition of plaques is different in renal compared to non-renal patients. Currently, there is a lack of data concerning the morphological characteristics of vascular structural changes and atherosclerosis in patients with different stages of renal failure. Therefore, the present study compares changes in different vessels obtained at cardiac surgery between patients with early and late stages of renal dysfunction and non-renal control patients.
Methods: 50 patients undergoing cardiac bypass surgery were devided into 3 groups:1. patients with S-creatinine <1.3 mg/dl (control group, n=24), 2. patients with moderately elevated S-creatinine (1.3-2.0 mg/dl, early CRF, n=14), 3. patients with established renal failure (S-creatinine >2.0 mg/dl or dialysis treatment, CRF, n=12). A. mammaria int., aorta, small subcutaneous arterioles and V. saphena were analysed using quan-titative morphometry and immunohistochemistry for markers of inflamma-tion (CRP, CD40, CD154, iNOS, eNOS, ICAM, VCAM). Then, univariate analysis and correlation analyses were performed.
Results: Media thickness and inflammation score of the A. mammaria int. and aorta was sig. higher in CRF patients than in controls. In contrast, significant inflammation of all vessels and calcification of the aortic media was already present in early CRF. Calcification of the aortic intima and of V. saphena was significantly more pronounced in CRF patients than in and controls. Of note, CaxP product correlated well with markers of inflammation, but not with calcification itself.
Conclusions: Early stages of CRF are associated with local upregulation of proinflammatory molecules in the vascular wall and calcification of the aortic media. These findings point to the importance of systemic and local microinflammation in CRF and may shed new light on the possibly overestimated role of the CaxP product for vessel calcification.

Transplantation 2

SO044 ★ TOLERANCE-INDUCING ENZYME INDOLEAMINE 2,3-DIOXYGENASE IS EXPRESSED IN PATIENTS AFTER COMBINED AUXILIARY LIVER AND KDINEY TRANSPLANTATION

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Introduction and Aims: Previous findings suggest that an auxiliary liver transplantation a few hours before a renal transplantation improves kidney graft survival in patients with multispecific HLA antibodies. To elucidate the mechanisms behind liver-induced graft acceptance we have investigated whether expression of tolerance-inducing genes coincides with graft acceptance and survival in these patients.
Methods: DNA microarrays were used to identify genes with altered expression levels in liver and kidney biopsies in one patient undergoing combined auxiliary liver and kidney transplantation. The expression levels of interesting genes, selected by bioinformatics methods, were validated by real-time PCR in samples from eight patients undergoing the same surgical procedure. The gene expression was also correlated to clinical outcome. To confirm that changes in gene expression is mirrored on protein level HPLC was used to detect enzyme-activity in patient sera.
Results: Expression of the tolerance-inducing gene indoleamine 2,3-dioxygenase (IDO) in the grafted organs was detected in the microarrays. The expression level of IDO was validated by real-time PCR in samples from 8 patients undergoing the same surgical procedure. IDO mRNA was upregulated 12-fold in the liver graft 4 hours after reperfusion and 90-fold in both liver and kidney after 1 week, whereas IDO-expression in the patient's own liver remained at the limit of detection similar to the values of the pre-transplant biopsies for all organs. Gene expression levels in the liver graft 4 h after reperfusion correlated with clinical outcome.
To verify IDO activity, the metabolite kynurenine and substrate tryptophan was measured in patient serum. Enzyme activity was measured as ratio between kynurenine and tryptophan. Preliminary results show an increase in IDO-activity after combined auxiliary liver and kidney transplantation (+102% 24h after transplantation, n=2), whereas IDO-activity decrease 24 h after regular kidney transplantation (-54%, n=5) compared to pre-transplant values.
Conclusions: We conclude that IDO is upregulated after auxiliary combined liver and kidney transplantation. This enzyme is tolerance-inducing and its expression correlates with clinical outcome in these patients, indicating a role for it in the liver-mediated acceptance of a renal graft in highly sensitized patients.

SO045 ❋ HEREDITARY FIBRINOGEN A α ALPHA-CHAIN AMYLOIDOSIS: PHENOTYPIC CHARACTERIZATION AND OUTCOME OF COMBINED LIVER AND KIDNEY TRANSPLANTATION

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Introduction and Aims: Genetic variants of fibrinogen A α alpha-chain cause the commonest type of hereditary renal amyloidosis (AFib) in the

U.K. Variant fibrinogen is produced in the liver, and renal transplants performed for AFib with end stage renal disease (ESRD) fail in 1- 7 years due to recurrent amyloidosis.

Methods: We report the clinical features and outcome of 20 patients with AFib who were assessed for combined liver and kidney transplant (LKT). Median age was 55 (49-69) years, 12 male; 19 had the E526V variant and one had R554L. Presentation was with proteinuria/nephrotic syndrome, and mode of diagnosis was renal biopsy in all.

Results: Renal and splenic amyloidosis was present in all patients on SAP scintigraphy. Coronary disease was identified in 60% of cases, and systemic vascular disease in 50%. Vascular atheroma excised at carotid endarterectomy in one case, contained abundant amyloid in the intima and thrombus, that on molecular analysis was wholly derived from variant fibrinogen. Cardial biopsies in a small number of E526V patients and in R554L, revealed amyloid deposits in the myocardium and blood vessels. Autonomic dysfunction was identified in 10 cases. Eight patients were declined LKT due to cardiovascular risks, 3 are listed for LKT, and the R554L patient is assessed for triple heart/liver/kidney transplant. Eight patients received LKT in the past 11 years, 6 of whom are well with good dual graft function at median fol-up of 43.7 (10-132) months. Biochemical studies confirmed that variant fibrinogen was eliminated from the plasma following LKT, and SAP scintigraphy has shown regression of visceral amyloid. Two long-term haemodialysis patients died postoperatively with dysautonomia and cardiovascular events.

Conclusions: AFib is a systemic amyloid disease with visceral, vascular, and neurological involvement. Combined liver and kidney transplantation in patients with AFib and ESRD eliminates the sole, hepatic source of variant fibrinogen, prevents amyloid recurrence, and facilitates regression of systemic amyloid deposits.

Cardiovascular disease is common in AFib; we are investigating the significance of amyloid deposition in the heart and vascular walls, along with the observed association of AFib with arterial disease.

SO046 IDENTIFICATION OF THE FACTORS THAT MIGHT PLAY A ROLE IN THE EXISTENCE OF ANTI-MICA ANTIBODIES IN THE GRAFT LOSS OF THE RENAL TRANSPLANT

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Introduction and Aims: MICA antibodies were associated with organ transplant rejection, having a strong homology with the HLA class I, but it has no role in antigen presentation. The objective of the study was to identify factors that were independently associated with the more presence of Anti-MICA antibodies in the graft loss of the renal transplant (RT).

Methods: A cross-sectional and a prospective study of RT patients were done in our hospital. Anti-MICA antibodies were tested by x-MAP Technology (LuminexÒ). Clinical variables: age and sex of donor and recipient, donor cause of death, recipient kidney disease, previous transplants, transfusions, preformed cytotoxic antibodies, HLA mismatches, cold ischemia time (CIT, recoded in <12 hours, between 13 and 19 hours, and >20 hours), renal function tested by serum creatinine (scr) and creatinine clearance (cCl), complications (delayed graft function (DGF), nephrotoxicity, acute rejection (AR), posttransplant diabetes mellitus (PTDM), infections, immunosuppressive drugs.

Results: A total of 96 RT were followed-up 5 years. 81% had first RT and 31 (32%) of them had anti-MICA antibodies. Donor: 41±17 years, 51% cerebral deceased of hemorragia/isquemia. Recipient: 48±11 years, 63% male, kidney disease: glomerulonephritis: 37%, polycystic kidney diseases: 13%, vascular nephropathy: 10%, tubulointerstitial nephritis: 8%, diabetes mellitus: 8%. CIT: 17±4 hours. Immunosuppression drugs: 28% induction therapy; CsA from the beginning 63%, Tacrolimus 43%, MMF 66%, Azathioprine 25%. Complications: 22% DGF; 19% nephrotoxicity; 12.5% AR; 12% PTDM. There were differences in the existence of previous RT (the patients with anti-MICA antibodies received more transplants 10/21 vs 8/57, p=0.026), in the grafts loss (the patients with anti-MICA antibodies lost more 5/26 RT vs 1/64, p=0.013) and CIT (p=0.003). The logistic regression was developed with the statistical significant variables in the univariate model to identify the variables that have independent effect on having anti-MICA antibodies.

Logistic Regression: anti-MICA antibodies as dependent variable

Variables	Sig.	Exp (B)	95,0% C.I.for EXP(B)	
			Lower	Upper
CIT	0.001	4,449	1,841	10,752
Graft lost	0.029	23,488	1,391	396,576
Previous RT	0.005	6,272	1,720	22,875

CIT: cold ischemia time.

Conclusions: Anti-MICA antibodies were associated with an increased frequency of graft loss. Previous RT, Graft lost and CIT were the variables that had independently effect on having anti-MICA antibodies. It is essential to keep investigating in the causes of the anti-MICA antibodies to obtain new possibilities of intervention in order to confirm the association of the immunological mechanism.

SO047 ♦ PERITUBULAR CAPILLARY AND VASCULAR MACROPHAGE INFILTRATION CORRELATES WITH MICRROVASCULAR DESTRUCTION AND WORSENS STEORID RESPONSE AND RENAL ALLOGRAFT OUTCOMES FOLLOWING C4D NEGATIVE ACUTE REJECTION EPISODES

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Introduction and Aims: We aimed to understand the influence of peritubular capillary (PTC) and vascular macrophage infiltration on steroid response and renal allograft outcomes after acute rejection (AR) episodes.

Methods: Seventy-nine patients with biopsy-proven AR in their first year after transplantation were included in the study. Thirty patients with normal first year renal allograft biopsies were also included in the study and used as a control group. All biopsies were C4d negative. Immunohistochemically we assessed the degree of macrophages (CD68) and HLA-DR-positive infiltrating cells in PTC's, glomeruli, and on vascular walls and tubules. In addition HLA-DR expression of PTC's was also evaluated. The decreasing intensity of peritubular capillary HLA-DR (PTC-DR) expression was accepted as the increasing degree of the destruction of PTC.

Results: Compared to control group AR cases showed significantly higher degree of macrophage and HLA-DR positive inflammatory cell infiltration in PTC's, glomeruli, and on vascular walls and tubules (P<0.001 for all). PTC destruction was significantly higher in AR cases than control group (p<0.001). PTC, glomerular and vascular macrophage infiltration showed significant correlation with PTC destruction and steroid response (p<0.001 for all).

Severity of PTC destruction with accompanying higher degrees of macrophage infiltration in PTC's, glomeruli and on vascular walls caused unresponsiveness to steroid therapy (p<0.001) and poor graft outcome (p<0.001). Five-year graft survival was 95%, 37% and 22% for cases with grade 0, 1 and 2 PTC macrophage infiltration respectively (P<0.001). In addition five-year survival was 80%, 36% and 1% for cases with grade 0, 1 and 2 vascular macrophage infiltration respectively (p<0.001).

Conclusions: In conclusion peritubular capillary and vascular macrophage infiltration are important predictors of steroid response and renal outcome following acute rejection in cases whom especially had negative C4d.

SO048 POLYOMAVIRUS INFECTION AND NEPHROPATHY: PROSPECTIVE STUDY IN KIDNEY TRANSPLANT PATIENTS

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Introduction and Aims: Nephropathy due to polyomavirus is usually diagnosed by renal biopsy after worsening of renal function. This is normally at an advanced stage of the disease and involves a four-fold increase in the risk of graft loss.
Aim: To study the early detection of the presence of BK and JC polyomavirus in urine by monthly real-time quantitative PCR assay.
Methods: The study included 76 kidney transplant recipients (49.2±13.2 years) from cadaveric donors (43.7±15.4 years) transplanted between August 2005 and July 2006 with a follow-up of one year. If the PCR in urine was positive PCR was performed in blood. If this was positive a renal biopsy was performed.
The patients received immunosuppressive therapy (93.4%) with tacrolimus, mycophenolate and prednisone. If PCR in blood was positive the immunosuppression was reduced.
Results: Viruria was positive in 31 patients (40.7%) and viremia in 7 (9.2%), 3 of whom (3.9%) developed nephropathy. After reduction of the immunosuppression there was no episode of acute rejection, the viruria became negative in 9 cases (29.0%) and the viremia in 3 (42.8%). Renal function (creatinine clearance, aMDRD) at one year was 49.2 ml/min/1.73 m^2 in the patients with nephropathy and 64.3 ml/min/1.73 m^2 in the others. One-year patient and graft survival was 96%. No patient lost the graft due to nephropathy.
Conclusions: The detection of BK and JC polyomavirus by the protocolized PCR enables early diagnosis of nephropathy and prevents associated graft loss, with good renal function one year later.

Cardiovascular diseases 1

SO049 SCREENING FOR RENAL ARTERY STENOSIS IN PATIENTS AFFECTED BY ISCHEMIC HEART DISEASE UNDERGOING CORONARY ANGIOGRAPHY

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Introduction and Aims: The clinical diagnosis of atherosclerotic renal artery stenosis (RAS) remains problematic because its clinical manifestations are not specific. Incidental diagnosis of RAS has become commonplace aided by technical advancement and by increasing number of endovascular procedures in elderly. The aim of our study was clinical detection and severity determination of RAS in a high cardiovascular risk population, referred for diagnostic or therapeutic cardiac catheterization.
Methods: From April 2006 to March 2007, all consecutive patients (pts) affected by ischemic heart disease undergoing non-emergent coronary angiography at a single institution were also evaluated for atherosclerotic RAS by renal arteriography. Clinical, laboratory and angiographic data were recorded.
Results: We studied 992 consecutive pts who underwent coronary and renal angiography. (age 64±10 yr, M 719 F 273, sCr 0.99±0.29 mg/dl, CrCl 84±31 ml/min, Diabetes 36%, Hypertension 86%, Hyperlipidemia 75%). 62 patients out of 992 (6%) had a significant RAS (>50%) and 746/992 (75%) had at least 1 coronary vessel involved (1 vessel in 278 cases, 2 vessels in 241 cases and 3 vessels in 158 cases, LMCA in 69 (7%). The presence of RAS was associated directly with the severity of CAD (P<0.001), age (P=0.001), serum creatinine (P<0.001), hypertension (P=0.03), pulse pressure (P=0.018), history of chronic renal insufficiency (P<0.001), history of peripheral vascular disease (P<0.001) and hyperlipidemia (P=0.057) and inversely with CrCl (P<0.001). RAS was slightly associated with diabetes (P=0.06), sex (P=0.13) and smoking (P=0.14). In a multiple logistic regression model, including all univariate correlates of RAS (i.e. with P<0.20), only serum creatinine [odds ratio (OR) (0.2 mg/dl increase): 1.41, 95% CI: 1.22-1.62, P<0.001], peripheral vascular disease (OR: 3.0% CI: 1.70-5.29, P=0.004), number of involved coronary vessels [OR (1 stenotic vessel increase): 1.41, 95% CI: 1.13-1.75, P= 0.001], male gender [OR: 0.41, 95% CI: 0.22-0.73, P= 0.003] and pulse pressure [OR (1 mmHg increase): 1.02, 95% CI: 1.00-1.03, P= 0.05] maintained an independent association with RAS. In a ROC curve analysis serum creatinine, peripheral vascular disease, number of involved coronary vessels, sex and pulse pressure jointly produced a ROC curve area of 0.78 (95% CI: 0.71-0.84, P<0.001). In this analysis, the contribution of the number of involved coronary vessels to identify RAS was very low (∼2%).
Conclusions: The prevalence of RAS in pts undergoing coronary angiography, not previously suspected of having RAS, is 6%. The number of stenotic coronary vessels does not seem to have a value greater than serum creatinine and PVD to identify patients at risk of RAS.

SO050 ♦ WARFARIN-INDUCED CALCIFICATION IN MICE – A NOVEL TOOL TO INVESTIGATE INTERACTIONS OF CALCIFICATION INHIBITORS

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Introduction and Aims: In patients with chronic kidney disease (CKD) mortality is largely increased due to accelerated vascular calcification. Extraosseous calcification is prevented by inhibitory proteins that act systemically (fetuin-A) or locally within the vessel wall (matrix gla protein, MGP). MGP requires vitamin K dependent gamma-carboxylation. We aimed to establish a model of warfarin-induced calcification in mice to facilitate analyses of calcification inhibitors using transgenic animals.
Methods: Twenty DBA/2 mice received diets with variable concentrations of warfarin (0.03, 0.3 and 3 mg/g) supplemented with vitamin K1 to prevent bleeding complications. Thirtytwo fetuin-A deficient mice on DBA/2 background were fed vitamin K deficient chow supplemented with either vitamin K1 or K2. All diets were continued for 4 weeks. Ex vivo analysis of vascular calcification was performed in murine VSMC cultured in warfarin containing (10 μM) and vitamin K supplemented medium (5 mM). Calcification was detected by flame atomic absorption spectrometry, the cresolphthalein method and histologically after von Kossa staining. Osteopontin, cbfa1 and MGP mRNA were quantified by RT PCR.
Results: We observed a warfarin mediated dose dependent increase in calcified area in von Kossa stained aortas from DBA/2 mice. As compared to mice fed standard chow, 3mg warfarin per g chow induced 5-fold higher calcification (p<0.001), 0.3mg warfarin induced a 4-fold higher calcification in the aorta (p<0.001). Calcium content in the heart was increased in mice receiving 3mg warfarin per g (16-fold, p<0.05). Likewise, ex vivo calcification of murine VSMC was enhanced by warfarin-mediated vitamin K antagonism (7-fold, p<0.001) and could be prevented by vitamin K1 and K2 supplementation (p<0.05 and p<0.001, respectively). In spontaneously calcifying Fet-$A^{-/-}$ mice a diet depleted in K1 or K2 failed to enhance overt calcification of the heart or aorta compared to Fet-$A^{-/-}$ mice on standard chow. There was no change in osteopontin, cbfa1 and MGP mRNA expression in these mice.
Conclusions: We established a model of warfarin-induced vascular calcification for the first time in mice, providing a fundament to dissect the relevance of vitamin K-mediated calcification in comparison to established calcification inhibitors using transgenic animals. The in vivo results were supported by induction of calcification in VSCM and subsequent inhibition by vitamin K in vitro. In addition tissue calcification in Fet-$A^{-/-}$ mice was not altered by selective vitamin K1 or K2 depletion.

SO051 ♦ VASCULAR CALCIFICATION IMPLIES DIFFRENTIAL GENE EXPRESSION PROFILE WITH DEREGULATION OF THE SFRPs FAMILY

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Introduction and Aims: Many studies have described the impact of vascular calcifications (VC) on morbidity and mortality in renal patients. VC process undergoes trough a complex mechanism that consists not in a simple precipitation of Calcium (Ca) and phosphate but in a regulation process similar to bone formation that involves the expression of mineralization-related genes. The aim of this study was to seek the differentially expressed genes between calcified and non calcified aortas in a rat model of CKD.
Methods: Rats were nephrectomized (7/8) and subsequently divided in two groups: a group fed with normal diet (0.6% phosphorus-P) and a group fed with high P diet (0.9%). In addition, a group of animals without nephrectomy and fed with normal diet was used as reference group. Rats were sacrificed by exsanguination after 8, 16 and 20 weeks of surgery (5 animals per group) and the abdominal aorta was extracted. Biochemical parameters were measured and Von Kossa staining of the aorta was performed to detect the presence of VC.
The 5 aortas of each group were pooled and RNA was extracted. cDNA was synthesized, and hybridized to arrays Affy RAE230. The arrays were normalized and modelled and hierarchical clusters were built to group samples and genes. The false discovery rate (FDR) algorithm was used to find the differentially expressed genes among the groups, and considering a fold change of 2 as threshold. The OveR score Analysis (ORA) method was also used to determine which molecular processes were altered across the arrays. The obtained results were confirmed by qRT-PCR.
Results: Only the group of animals fed with high P during 20 weeks (calcified group) developed VC, assessed by Von Kossa staining. Moreover, this group had significantly increases in serum Ca and iPTH and a increase in serum P. The unsupervised hierarchical cluster of samples showed that the arrays of the non calcified groups clustered together and the separated from the calcified group. Genes with time-dependent changes were taken as candidates, and we found 31 genes that reach the maximum repression or over expression in the calcified group compared to the non calcified group. We found a decrease in Tropomyosin and Elastin, and an increase in bone related genes such as Cathepsin K. Interestingly; 4 members of the secreted related frizzled proteins family (SFRPs) were up-regulated. SFRPs family is known as inhibitor of the wnt pathway, which is involved in the bone metabolism. All these findings were confirmed by qRT-PCR. A higher level analysis (ORA), determined that the cytoplasmic scaffold, GTPases activity regulation and the oxidative stress pathway were significantly altered in the calcified group.
Conclusions: Up to twenty weeks were necessary to observe aortic VC in 7/8 nephrectomized rats fed with high P diet. Calcified aortas showed a different gene expression profile with over- expression in bone related genes and members of the SFRP family and repression of muscle related genes. Moreover, molecular pathways such as oxidative stress were significantly altered.

SO052 ★ THE IMPACT OF CORONARY ARTERY CALCIFICATION ON MORTALITY IN HEMODIALYSIS PATIENTS

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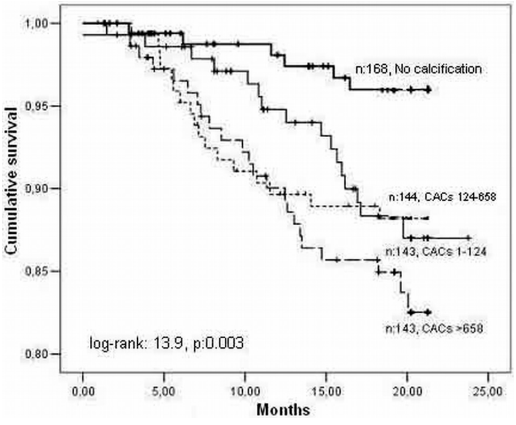
Introduction and Aims: Coronary artery calcification (CAC) has been suggested as a non-traditional risk factor to explain incredibly increased rate of cardiovascular disease (CVD) in hemodialysis (HD) patients. However, there is limited data about the impact of CAC on survival. We investigated the effect of CAC on survival in a large cohort of HD patients and the relationship between CAC and calcification-related proteins.
Methods: Between September-November 2005, 598 prevalent HD patients underwent multi-slice computerized tomography to quantify CAC; CAC scores were measured by the same radiologist (Agatston method). All subjects (mean age 58±14 years, HD duration 55±46 months) were prospectively followed for 18 months; all-cause mortality was evaluated. Baseline demographical, clinical and time-averaged laboratory data were assessed. In a subgroup of patients (n: 276), whose clinical characteristics were similar with whole study population, fetuin-A, osteoprotegerin (OPG) and matrix gla protein (MGP) levels were measured by ELISA in serum samples collected at the time of CAC assessment. Predictors of all-cause mortality were analyzed by Cox regression analysis. Cumulative survival was estimated by Kaplan-Meier method.
Results: The frequency of CACs was 27.7%, 22.5%, 19.2% and 30.6% for scores 0, 1-100, 101-400 and >400, respectively. Serum OPG levels were increased with age (r: 0.40, $p<0.001$) and positively correlated with dialysis duration (r:0.16, $p<0.01$). The serum OPG levels were positively correlated with CACs (r:0.24, $p<0.001$) and increased by CACs tertiles (9.2±5.9, 11.2±6.2 and 11.7±5.4 pg/L in the 1st, 2nd, and 3rd tertile, respectively, $p<0.001$). Serum fetuin-A levels were negatively correlated with age (r: -0.17, $p<0.05$). Neither fetuin nor MGP was correlated with CACs.
During a mean follow-up of 18±5 months, mortality rate was 10.5% (n:62). Mean CACs was higher in those who died compared with patients alive (1343±2329 vs 525±1018, $p<0.001$). When compared to the alive patients, age (65±13 vs 58±13 years, $p<0.001$), prevalence of diabetes (39% vs 22%, $p<0.01$), inter-dialytic weight gain (2.5±0.9 vs 2.2±0.9 kg, $p<0.05$) and CRP levels (2.9±3.2 vs 1.5±1.5 mg/dl, $p<0.001$) were higher in patients who died.
Mortality significantly increased by CACs tertiles (Figure). In Cox-regression analysis, CACs, age, IDWG, body-mass index and CRP were independent predictors for mortality ($p<0.001$). Risk-adjusted RR was 2.6-fold for severe CACs (last tertile; score>658) when compared with score 0.



Conclusions: This prospective study, which is the largest one in the literature, demonstrates that CAC is an important independent predictor for all-cause mortality in HD patients.
Disclosure: This study was supported by Fresenius Medical Care.

SO053 ANTI-HYPERTENSIVE AGENTS (AHAs) AND CLINICAL OUTCOMES AMONG INCIDENT HEMODIALYSIS PATIENTS: THE DIALYSIS OUTCOMES AND PRACTICE PATTERNS STUDY (DOPPS)

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Introduction and Aims: Use of angiotensin receptor blockers (ARBs) and beta blockers (BBs) has been associated with lower mortality in recent studies of prevalent HD patients, but there is concern that their use may hasten loss of residual renal function (RRF) among incident HD patients.
Methods: We studied 8,176 patients entering the DOPPS I and II studies within 30 days of starting HD. This sample included >400 facilities in 12 countries. Patients were classified as taking one or more AHAs by class at study entry: BB, renin angiotensin system [RAS] inhibitors (ARB or ACE

inhibitor), calcium channel blocker (CCB), peripheral blocker/vasodilator (PB/V), central antagonist (CA), diuretics. Mortality and time to loss of RRF (defined as >200 ml urine/24 hours) were examined using Cox models adjusted for each AHA class concurrently as well as for age, sex, race, 14 summary comorbid conditions; stratified by country and phase. Mortality models also adjusted for SBP, RRF, and albumin. Treatment was characterized at the patient level (Y/N) and facility level (% of incident HD patients prescribed each AHA class at the facility, adjusted for case mix).
Results: Use of BB, RAS, and diuretics increased significantly between DOPPS I and DOPPS II (p<0.05), from 27% to 33%, 28% to 37%, and 32% to 43%, respectively. Use of CCB significantly decreased over the same period from 52% to 47%. Only 21% of this sample was not taking any type of AHA. The majority of patients were taking more than one AHA. The most common combinations were BB/CCB, BB/RAS, and RAS/CCB. In Cox models, RAS inhibitors were associated with significantly lower mortality in patient- and facility-based models and with RRF preservation in the patient-based model, with p=0.10 in the facility-based model. Associations with mortality and RRF did not vary according to type of RAS inhibitor (ACEI or ARB). Diuretics were associated with preservation of RRF, but this may be because RRF is an indication for diuretic use and diuretics may maintain urine output without preserving renal clearance.

AHA Medication	All-Cause Mortality (n=8,176)				Loss of RRF (n=1,254)*			
	Patient Based (Med vs. No)		Facility Based (per 10% more)		Patient Based (Med vs. No)		Facility Based (per 10% more)	
	HR	p	HR	p	HR	p	HR	p
Beta Blockers	*0.89*	*0.06*	1.02	0.45	0.96	0.67	1.03	0.42
RAS Inhibitors	***0.87***	***0.05***	***0.95***	***0.01***	***0.76***	***0.004***	0.95	0.10
Calcium Channel Blocker	***0.88***	***0.04***	1.02	0.55	1.04	0.62	0.97	0.45
Peripheral Blocker/Vaso	0.93	0.34	1.02	0.66	0.99	0.92	0.95	0.26
Central Antagonist	0.91	0.31	0.99	0.85	1.04	0.78	*0.91*	*0.06*
Diuretic (RRF adjusted)	0.94	0.37	0.98	0.22	*0.86*	*0.06*	***0.95***	***0.003***
Diuretic (no RRF adjust)	0.94	0.25	***0.96***	***0.02***				

*Among patient with RRF at baseline.

Conclusions: Among incident HD patients, use of RAS (ACEIs and ARBs) was associated with improved survival and possibly with preservation of RRF, accounting for baseline SBP. These data generally support the use of these agents and are inconsistent with the concern that their use may hasten loss of RRF among incident HD patients. No other AHA classes were associated with a survival benefit or loss of RRF in both patient- and facility-based models.
Disclosure: The DOPPS is supported by research grants from Amgen, Inc. and Kirin Pharma Co., Ltd., without restrictions on publications.

SO054 CARDIAC NATRIURETIC PEPTIDES REFLECT LEFT ATRIAL VOLUME (LAV) AND CAPTURE LONGITUDINAL CHANGES IN LAV IN ESRD PATIENTS

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Introduction and Aims: Left atrial volume (LAV) has recently emerged as a prognostic factor which can be used for risk stratification (*J Hypertens 2006; pp. 1173*) and risk monitoring (*JASN 2007: pp. 1316*) in patients with end stage renal disease (ESRD). Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are two biomarkers which reflect atrial and ventricular pressures and left ventricular mass but the relationship of these peptides with LAV are still scarcely characterised.
Methods: We have therefore tested the relationship between ANP and BNP with LAV and with LAV changes over a follow-up of 17±2 months. This longitudinal study was performed in a cohort of 189 ESRD patients (age: 59±16 yrs; 109 M and 80 F).
Results: LAV was significantly higher in dialysis patients than in healthy subjects (P<0.001). On univariate analysis, both plasma ANP (r=0.57, P<0.001) and BNP (r=0.59, P<0.001) were strongly and directly related to baseline LAV and these associations remained substantially un-modified (ANP-LAV link: β=0.54, P<0.001; BNP-LAV link: β=0.54, P<0.001) in hierarchical models adjusting for Framingham risk factors (age, sex, smoking, diabetes, cholesterol, systolic pressure), background CV complications, risk factors peculiar to ESRD (haemoglobin and calcium phosphate product) and emerging risk factors (CRP and homocysteine). During the follow-up there was a significant increase in LAV (from 10.4±5.0 ml/m$^{2.7}$ to 11.6±5.6 ml/m$^{2.7}$, P<0.001). Changes in LAV (as adjusted for the corresponding baseline value) were directly and significantly related to plasma ANP (r=0.20, P=0.006) and BNP (r=0.23, P=0.002) and these associations held true in multiple regression models (ANP- Δ LAV link: β=0.18, P=0.03; BNP- Δ LAV link: β=0.19, P=0.02) including the same set of confounders considered above.
Conclusions: Cardiac natriuretic peptides are independently related to LAV and predict the worsening in LAV in patients with ESRD. Because an increased LAV underlies diastolic dysfunction and/or volume expansion, i.e. potentially modifiable risk factors, the measurement of the plasma concentration of ANP and BNP might be useful for risk stratification and for guiding treatment in dialysis patients.

Experimental pathology

SO055 PDGF-C ACCELERATES GLOMERULAR CAPILLARY REPAIR IN EXPERIMENTAL GLOMERULONEPHRITIS

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Introduction and Aims: Glomerular endothelial cell injury is a key component of a variety of glomerular diseases. Factors involved in glomerular endothelial cell repair are promising targets for the treatment of such glomerular diseases. Platelet-derived growth factor (PDGF)-C has pro-angiogenic properties, however, nothing is known about such functions in the kidney. We hypothesized that administration of exogenous PDGF-C accelerates glomerular repair mechanisms in rats with anti-Thy 1.1 glomerulonephritis, a model that is characterized by an early glomerular mesangial and endothelial cell injury.
Methods: Rats were treated with active recombinant human PDGF-C (n=15, i.p. via osmotic pumps) or PBS control (n=15) from d1 until d4 following anti-Thy 1.1 induction. All rats were sacrificed at d5.
Results: PDGF-C treatment significantly improved glomerular capillary repair. Glomerular mesangiolysis (-30%, PDGF-C vs. control, p<0.01), the frequency of microaneurysms (-61%, p<0.01) and glomerular expansion (-10%, p<0.05) were significantly ameliorated in rats treated with PDGF-C. The histological findings were paralleled by a significant functional improvement in PDGF-C treated rats (reduction of the number of rats doubling their blood urea nitrogen). PDGF-C treatment specifically improved glomerular endothelial cell recovery without affecting mesangial cell repair. Glomerular endothelial area (JG12 staining; +32%, p<0.01) as well as glomerular endothelial cell proliferation (endothelial mitoses, +82%, p<0.01; PCNA/JG12 double staining, +23%, p=0.055) were significantly increased in PDGF-C treated rats, while mesangial cell proliferation and -activation (mesangial mitotic figures, PCNA/a-smooth muscle actin double staining) did not differ between groups. The pro-angiogenic PDGF-C effects were independent of VEGF-A, since we detected no differences in serum or glomerular VEGF-A protein levels or glomerular VEGF-A mRNA expression. PDGF-C infusions did not affect body weight or blood pressure.
Conclusions: These results identify PDGF-C as a novel, potent pro-angiogenic factor accelerating glomerular capillary healing in experimental glomerulonephritis.

SO056 ★ IMATINIB (IMB) AND PLATELET-DERIVED GROWTH FACTOR RECEPTOR (PDGFR) β SPECIFIC INHIBITION ATTENUATE CRYOGLOBULINEMIC MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS (MPGN) IN MICE

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Introduction and Aims: IMB is a receptor tyrosine kinase inhibitor which blocks the activity of c-Abl, c-Kit, and PDGFRs. We tested the protective effects of imatinib and PDGFRβ blockade in thymic stromal lymphopoietin (TSLP) transgenic (tg) mice, a model of cryoglobulinemic MPGN in which some glomerular (GLM) manifestations are likely a consequence of PDGFRβ engagement.
Methods: Groups of TSLP tg mice and wild type controls were treated with IMB or vehicle daily by intraperitoneal injection. Treatment (Tx) was started at age 21-day (early-onset group (EOG)) or at age 90-day (late-onset group (LOG)) and was continued for 4 weeks, when mice were then sacrificed. The Rat anti-PDGFRβ monoclonal antibody (APB5) or control rat IgG (r2A) were administered on alternate days at age 90-day for 4 weeks. Routine histology, immunohistochemistry and in situ hybridization were performed. B cell subpopulations in spleen were examined by FACS.
Results: The total amount of circulating cryoglobulins, serum immunoglobulins (IG) and spleen weight was significantly reduced after both early- and late-onset of treatment with IMB, whereas APB5 Tx did not change these parameters compared to controls. IMB-treated TSLP tg mice exhibited a significant reduction of renal pathological findings including reduced extracellular matrix (ECM) (% GLM tuft area (GTA) occupied by silver stained matrix: EOG: 26.67±2.34 vs 15.90±1.48%, $P < 0.001$; LOG: 14.27±1.08 vs 6.79±0.71%, $P < 0.001$), mesangial cell activation (% GTA occupied by αsmooth muscle actin expressing cells: EOG: 8.89±1.95 vs 2.00±0.40%, $P < 0.001$; LOG: 1.87±0.14 vs 1.06±0.16%, $P < 0.01$). Tx with IMB significantly increased GLM macrophage infiltration in TSLP tg mice (GTA occupied by Mac-2 expressing cells: EOG: 8.20±2.01 vs 45.90±5.79 μm^2, $P < 0.01$; LOG: 61.36±11.89 vs 142.33±51.98 μm^2, $P < 0.05$). The analysis of B cells in spleen revealed that IMB reduced B220+ B cell number as well as follicular mature (FM) B cell number (B220+ B cell: 130.62±20.11 vs 62.31±9.84×10^6, $P < 0.05$; FM B cell: 67.32±13.70 vs 27.29±6.81×10^6, $P < 0.05$). Tx with IMB did not affect GLM expression of PDGFRβ. Tx with APB5 had beneficial effects on renal histology in TSLP tg mice with significantly decreased GTA (2505±76 vs. 2770±142 μm^2, $P < 0.05$), GLM cell number per GTA (27.2±0.69 vs. 29.2±1.0, $P < 0.05$), and ECM (% GTA occupied by trichrome stained matrix: 19.0±1.7 vs. 24.7±2.0%, $P < 0.05$) compared to controls.
Conclusions: These results provide the evidence that IMB has potential to attenuate cryoglobulinemic MPGN in human both during the initiation of injury and after the mesangial matrix accumulation characteristic of chronic injury is well established. Furthermore, these mechanisms are attributable to not only PDGFR inhibition but also the inhibition of c-Abl and/or c-Kit that were known to be involved in B cell development.

SO057 ★ DYSREGULATION OF NFκB IN PODOCYTE DISEASES: ROLE OF cmip

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Introduction and Aims: Idiopathic nephrotic syndrome (INS) is a podocyte disease in which the progression of cellular injuries is associated with podocyte depletion and appearance of glomerulosclerosis. Inhibition of NFκB activity has been shown to be associated with podocyte apoptosis and progressive glomerulosclerosis. We found that cmip, a new PH domain- and LRR-domain-containing protein, is up-regulated in podocytes of patients with INS. Targeted over-expression of cmip in mice podocytes induces nephrotic proteinuria. The lack of detection of cmip in normal kidney suggests that cmip is strongly inhibited in physiological situations. We aimed to study the physiological regulation of cmip and to analyze the functional consequences of up-regulation of cmip in pathological conditions.
Methods: The cmip promoter was isolated by combining 5'RACE and PCR amplification of genomic DNA sequences upstream of the 5'UTR adjacent to the coding sequence of cmip. Luciferase activity and preparation of nuclear fractions for EMSA experiments were performed by standard techniques. Differentiated podocytes were incubated overnight with 5% decomplemented and filtrated sera of children with normal and minimal change nephrotic syndrome (MCNS). The cmip protein was over-expressed in stably transfected podocytes. Transgenic mice over-expressing cmip under the control of the nephrin promoter were generated in order to restrict the transgene expression to podocytes. Functional consequences of cmip over-expression in podocytes were analyzed by immunoprecipitation, luciferase activity assays, western blotting, immunofluorescence staining and caspase 3 activity assays.
Results: We identified one responsive element that displays high NFκB DNA binding on cmip promoter and we showed that NFκB strongly inhibits the transcriptional activity of cmip gene. Conversely, cmip interacts with NFκB p65 and represses NFκB activity. Moreover, overexpression of cmip in stably transfected murine podocytes induces cytoskeleton disorganization, cell detachment and triggers caspase 3 activity. Interestingly, we observed that incubation of murine podocytes with MCNS relapse serum but not with the remission serum or normal serum induces an up-regulation of cmip and a down-regulation of NFκBp65 protein expression. Finally, we found that cmip transgenic mice also exhibited a profound down-regulation of NFκBp65 protein expression associated with activation of pro-apoptotic signals.
Conclusions: Altogether, these results demonstrate that cmip and NFκB are mutually antagonistic. In physiological conditions, NFκB inhibits cmip transcription and promotes podocyte survival. By contrast, in pathological conditions such as MCNS, cmip is induced, represses NFκB activity and activates pro-apoptotic signals. In conclusion, these results suggest that negative crosstalk cmip/NFκB plays a critical role in pathophysiology of podocyte diseases.

SO058 ★ ACTIVATION OF TOLL-LIKE RECEPTORS EPRESSED BY PODOCYTES LEADS TO INDUCTION OF CC AND CXC CHEMOKINES

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Introduction and Aims: Toll-like receptors (TLRs), as pathogen recognition receptors, classically detect evolutionary conserved danger signals, e.g. structural motifs of bacterial or viral origin. TLRs are known to be predominantly expressed by leukocytes belonging to the innate immune system. However, previously we could show functional expression of TLR3 by mesangial cells, activation of TLR3 led to an induction of proinflammatory chemokines (Am J Path 2006). The aim of the present study was to examine the TLR expression pattern of podocytes under basal conditions, or after treatment with proinflammatory cytokines (IL-1β, IFN-γ, TNF-α), respectively. Thereafter, TLR-dependent chemokine expression was analyzed after stimulation of the cells with potential exogenous or endogenous TLR ligands. To prove specificity of TLR-mediated effects siRNAs blocking TLR expression were developed.
Methods: In murine podocytes we investigated the expression of TLR1-9 and 11 under basal conditions as well as after incubation with IL-1β, IFN-γ and TNF-α alone or in combination by quantitative real-time PCR. Dependent on the availability of specific antibodies TLR expression was analyzed on protein level too. Subsequently chemokine expression was determined under basal and stimulatory conditions for different incubation periods (2h to 48h), in the absence or presence of TLR-specific siRNA.
Results: Under basal conditions, a significant expression of TLR1 to 7, 9 and 11 and a low expression of TLR8, 12 and 13 was detected. After incubation of podocytes with a combination of IL-1β, IFN-γ and TNF-α, a different expression pattern was detectable. Examination of chemokine expression after treatment with the exogenous TLR-ligands peptidoglycan, poly I:C RNA or Lipid A, respectively, revealed a significant upregulation of the chemokines CCL2, CCL5, CCL7, CXCL1, and CXCL5. Specificity of TLR signalling could be proven by downregulation of single TLRs via

specific siRNAs. Investigating potential endogenous TLR ligands, fibrinogen induced a massive chemokine induction, whereas heat shock proteins had only a minor effect on podocytes.
Conclusions: Our experiments showed a specific expression pattern of functionally active TLRs on murine podocytes. Since their pivotal role will not be the local reaction to bacterial or viral infection, a transmission of endogenous danger signals might be hypothesized. The activation of toll-like receptors and a subsequent chemokine expression may play a role during the initiation of glomerular damage.

SO059 CD2AP/CIN85 PROTEIN BALANCE REGULATES THE SLIT-DIAPHRAGM TURNOVER

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Introduction and Aims: The adaptor molecules CD2AP and CIN85 influence the activation and maintenance of intracellular signaling cascades PI3K/AKT and ERK1/2. Disruption of these signaling pathways lead to increased cell death in podocytes in vivo and in vitro We have previously shown that the sensitive balance of CD2AP/CIN85 plays a general role for the activation of the most Receptor-Tyrosine-Kinases (RTK) in podocytes. The interaction of CD2AP with Nephrin and Podocin is well established, however the interaction with CIN85 and the involvement of CIN85 in the physiological turnover of the slit-diaphragm has not been described. The aim of our study is to clarify the effect of the CD2AP/CIN85 balance on the integrity of the slit-diaphragm-proteins Nephrin and Podocin.
Methods: We analyzed expression of Nephrin and Podocin in CD2AP+/+ and CD2AP-/- podocytes at different points of cellular differentiation. By immunoprecipitation- and immunofluorescence-assays we studied interaction of CIN85 with Nephrin and Podocin and Ubiquitination-profiles of both proteins. Furthermore we overexpressed CIN85 by transient gene delivery in vivo. Ultimately Expression of CIN85 on human kidney tissues of several glomerular diseases was analyzed.
Results: With time of differentiation expression of Nephrin and Podocin is decreased whereas expression of CIN85 is simultaneously increased in CD2AP-/- podocytes. We can show an inducible binding of CIN85 with Nephrin and Podocin after RTK-stimulation. This leads to ubiquitination and internalisation of Nephrin and Podocin in CD2AP-/- podocytes after RTK-stimulation. Transient gene delivery and overexpression of CIN85 leads to significant proteinuria in CIN85-injected mice compared to control-vector-injected mice. Furthermore expression of CIN85 is upregulated in several human glomerular diseases.
Conclusions: Our results indicate that the CD2AP/CIN85 balance is involved in the regulation of the slit-diaphragm in physiological and pathophysiological conditions.