

**O1. An early biomarker of acute rejection after renal transplantation detectable in urine**

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**Background**

Acute rejection is a serious complication of renal transplantation. Early and rapid diagnostics is important in order to initiate treatment or to identify other causes like acute tubular necrosis (ATN), drug toxicity and infection. With present-day immunosuppression the clinical manifestations of rejection have changed. Often, there are no local symptoms with insidious, low-level graft dysfunction. Biopsy is performed at the moment that graft dysfunction is already present, which is rather a late moment in the process of ongoing inflammation. Whatever the immunological nature of different kinds of rejection is, an early rapid and non-invasive diagnostic test may lead to prompt initiation of treatment and hopefully a better graft outcome.

**Methods**

We collected 90 urine samples from 33 renal allograft recipients with either acute rejection, ATN, primary CMV infection or stable function. Sixteen had biopsy-confirmed acute rejection, 5 had biopsy-confirmed ATN, 5 primary CMV and 7 stable function. In 5 subjects with acute rejection we succeeded in collecting urine samples weekly before the clinically manifest rejection, i.e. timely before the biopsy. Total RNA was isolated from urine-cell pellets, quantified and reverse transcribed to complementary DNA. By semi-quantitative real-time PCR (Taqman) detecting mRNA for granzyme A, perforin and granzyme B, we studied the correlation between relative mRNA expression levels of these cytotoxic proteins and the graft status. Results are normalized to the internal control 18S rRNA and expressed relative to the respective values of the parameters in LAK cells.

**Results**

The relative expression levels of granzyme A (GrA) mRNA, perforin mRNA and granzyme B (GrB) mRNA, which encode cytotoxic proteins, were higher in the urinary cells from the 16 patients with a biopsy-confirmed episode of acute rejection than in the 7 recipients with stable graft function and than in the 5 recipients with biopsy confirmed ATN (GrA, mean  $\pm$  SD; acute rejection:  $0.11 \pm 0.2$ , primary CMV:  $0.06 \pm 0.07$ , ATN:  $0.00 \pm 0.00$  stable:  $0.00 \pm 0.00$ ,  $P < 0.001$ ), (GrB, mean  $\pm$  SD; acute rejection:  $0.04 \pm 0.06$ , primary CMV:  $0.01 \pm 0.02$ , ATN:  $0.00 \pm 0.00$  stable:  $0.01 \pm 0.01$ ,  $P < 0.001$ ) and (perforin, mean  $\pm$  SD; acute rejection:  $0.16 \pm 0.19$ , primary CMV:  $0.06 \pm 0.04$ , ATN:  $0.01 \pm 0.02$ , stable:  $0.02 \pm 0.02$ ,  $P < 0.001$ ). Timely obtained sequential urine samples from 5 patients showed an early rise in GrA mRNA level preceding the rise in serum creatinine. This could only be detected for GrA messenger RNA and not for GrB and perforin, which renders GrA-mRNA in urine a highly sensitive marker for acute rejection. As yet, it can serve as an early alarm signal to perform a biopsy and confirm the diagnosis. If confirmed in a larger group of patients, a renal biopsy to exclude acute rejection in recipients with ATN can be withheld if no GrA-mRNA is present in their urine. Extension of this study to determine the predictive and prognostic value of GrA-mRNA in urine after renal transplantation is currently performed.

**Conclusion**

Measurement of mRNA encoding granzyme A in urinary cells offers a highly sensitive early non-invasive means of diagnosing acute rejection which may precede the rise in serum creatinine. Detection of mRNA for GrA in urine of renal transplant recipients should prompt the performance of a renal biopsy. Next to confirmation in a larger cohort of transplant recipients, the predictive and prognostic value for rejection outcome needs to be further investigated.

**O2. Mutations in the gene encoding the basal body protein RPGRIP1L, a novel nephrocystin-4 interactor, cause Joubert syndrome**

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**Background**

Cerebello-Oculo-Renal-Syndromes are a group of autosomal recessive disorders that involve abnormalities in the brain, retina and kidney. One of the syndromes that is part of this group is Joubert syndrome that is characterised by a typical hind brain malformation known as the "molar tooth sign", which results from cerebellar vermis hypoplasia. To date, four genes are known to be involved in Joubert syndrome, and most of the protein products are associated to cilia. Our aim was to elucidate the role of RPGRIP1L in the molecular mechanisms of Cerebello-Oculo-Renal-Syndromes.

#### **Methods**

SNP and microsatellite marker genotyping and DNA sequencing were used to screen RPGRIP1L for mutations; reverse transcriptase (RT) PCR was used to determine the expression of RPGRIP1L in different tissues; coimmunoprecipitation, GST-pulldown, yeast two-hybrid and colocalisation experiments with recombinant fluorescent proteins were used to test the interaction between RPGRIP1L and nephrocystin-4; immunohistochemistry, preembedding immunoelectron microscopy and immunocytochemistry were used to determine the localisation of RPGRIP1L in tissues and cells.

#### **Results**

We examined the role of RPGRIP1L, the protein product of RPGRIP1L that is located on chromosome 16q12.2. Homology searches of EST databases with RPGRIP1 (RPGR interacting protein 1), a ciliary protein involved in congenital blindness, revealed RPGRIP1L as a single homologue. We found that RPGRIP1L interacts via its C2-domains with nephrocystin-4 and that mutations in the nephrocystin-4 gene (NPHP4) that are known to cause Senior Løken syndrome disrupt this interaction. RPGRIP1L is ubiquitously expressed and its protein product localizes to basal bodies in brain, retina and kidney. Therefore, we selected RPGRIP1L as a candidate gene for CORS. We identified homozygous frameshift and splice site mutations in two families with typical Joubert syndrome and compound heterozygous nonsense and missense mutations in a third family. These findings clearly demonstrate that RPGRIP1L is associated with Joubert syndrome. Interestingly, one of the patients had postaxial polydactyly and encephalocele, which resembles the phenotype of Meckel Gruber syndrome, suggesting that RPGRIP1L could be involved in overlapping ciliary disorders.

#### **Conclusion**

Our work identifies RPGRIP1L as a novel gene for Joubert syndrome, and establishes a central role for cilia and basal bodies in the pathophysiology of this disorder.

### **O3. A cyclophosphamide-based treatment strategy reduces the risk of ESRD in patients with idiopathic Membranous Nephropathy. A Nationwide Survey in the Netherlands.**

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#### **Background**

Use of immunosuppressive therapy in patients with iMN is debated. From 1991 onward, we have advocated a restrictive treatment strategy in our University Hospital and regional referring hospitals. We advised the use of immunosuppressive therapy, consisting of a combination of steroids and oral cyclophosphamide for 12 months, in patients with iMN at high risk for ESRD.

#### **Methods**

Primary renal diagnosis of all patients who start renal replacement therapy in the Netherlands is registered in the RENINE database. We studied the incidence of ESRD due to iMN in the Netherlands in the period 1991-2005. We mailed a questionnaire to all nephrology centers who entered a patient with iMN in the RENINE database after 2000.

#### **Results**

The introduction of the cyclophosphamide-based treatment strategy in our region resulted in a significant 75% reduction in the incidence of ESRD in patients with iMN as compared to the increasing incidence of ESRD in other parts of the Netherlands. Response rate to the questionnaire was 65%. There were 45 patients (34 M, 11 F), with a mean age at diagnosis of  $49 \pm 17$  yrs and a median serum creatinine of  $138 \mu\text{mol/l}$  (range 60-1798). Overall, only 22 patients (49%) had been treated with immunosuppressive therapy, consisting of prednisone monotherapy in 7. Only five patients had received cyclophosphamide for at least 3 months.

#### **Conclusion**

A cyclophosphamide-based restrictive treatment policy reduces the risk of ESRD in iMN. The questionnaires reflect the differences in opinion on the optimal treatment of high-risk patients with iMN.

#### **O4. Beta-2-microglobulin is superior to N-acetyl-beta-glucosaminidase in predicting prognosis in idiopathic membranous nephropathy.**

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##### **Background**

An accurate prediction of prognosis in patients with idiopathic membranous nephropathy (iMN) would allow restriction of immunosuppressive treatment to patients who are at highest risk for end stage renal disease (ESRD). Several markers of proximal tubular cell injury have been used as predictors of prognosis. In this study we compared the accuracy of urinary beta-2-microglobulin (U-beta2m) and N-acetyl-beta glucosaminidase (U-beta-NAG) in predicting renal insufficiency and remission rates.

##### **Methods**

57 patients with iMN (38M, 19F; age 48 ±16 yr), a nephrotic syndrome, and a serum creatinine level <135 µmol/l were studied prospectively. At baseline, a standardized measurement was carried out to determine renal function and protein excretion. The end point renal death was defined as a serum creatinine exceeding 135 µmol/l or a rise of serum creatinine of >50%. Remission was defined as a proteinuria < 2.0 g/day with stable renal function.

##### **Results**

Mean follow-up was 80±36 months. Mean serum creatinine concentration was 88±20 µmol/l, serum albumin 24±5 g/L and proteinuria 8.9±4.8 g/24h. Thus far, 28 (49%) patients have reached the predefined endpoint of renal death. Multivariate analysis identified U-beta2m as the strongest independent predictor for the development of renal insufficiency. Sensitivity and specificity were 81 and 90% respectively for U-beta2m (threshold value 6.1 microg/g cr), and 74 and 81% respectively for U-beta-NAG (threshold value 23.4 microg/g cr). Overall remission rate was 44%. A remission occurred in 78% of patients with low U-beta2m and in 14% of patients with high U-beta2m, and respectively in 71% of patients with low U-beta-NAG and 21% of patients with high U-beta-NAG.

##### **Conclusion**

Although both U-beta2m and U-beta-NAG predicted progression and remission in iMN, U-beta2m was more accurate. High specificity in predicting prognosis should be pursued to avoid unnecessary immunosuppressive therapy. We therefore conclude that U-beta2m is superior to U-beta-NAG in predicting prognosis in patients with iMN.

#### **O5. Systemically administered hematopoietic stem cells migrate to ischemic damaged kidney independent of the SDF-1alpha/CXCR4-axis**

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##### **Background**

Ischemia/reperfusion injury (IRI) is the major initiator of ARF and is caused by a transient drop in blood flow to the kidney. Depending on the severity of injury, the kidney has the capacity to restore itself. Hematopoietic stem cells (HSC) are thought to be involved in this reparative process, however, the mechanism of HSC trafficking to IRI kidneys remains unclear. SDF-1alpha and its receptor CXCR4 are identified as the central signaling axis regulating the homing of HSC to the bone marrow and injured organs such as heart and liver. Therefore, we hypothesized that SDF-1alpha and CXCR4 regulate the migration of HSC to IRI kidneys. To test this we manipulated the SDF-1alpha/CXCR4-axis after which HSC homing to the IRI kidney was evaluated.

##### **Methods**

Bone marrow was isolated from WT mice and sorted for c-kit<sup>high</sup> cells, resulting in a population of highly purified HSC. HSC were labeled with cell-tracker. Acceptor mice were subjected to unilateral ischemia and received directly after reperfusion 0.55-0.60x10<sup>6</sup> HSC iv. One day after IRI mice were sacrificed and kidneys were removed. The presence of injected HSC in kidneys was determined by means of FACS analysis and microscopy. To determine the effect of SDF-1alpha and CXCR4 on HSC migration, mice received either recombinant SDF-1alpha intrarenally or neutralizing anti-SDF-1alpha iv followed by iv injection of HSC, or HSC neutralized with anti-CXCR4 iv. Another group of mice was subjected to bilateral ischemia. Directly after reperfusion they received intrarenal injections of SDF-1alpha (left) or saline (right), followed by iv injection of HSC. Kidneys were analyzed as described before.

##### **Results**

In the unilateral IRI model, one day after IRI and iv administration of labeled HSC, these cells could be detected in the tubuli and interstitium of the kidney. Importantly, the amount of HSC in the ischemic damaged kidney was markedly higher ( $p < 0.05$ ) compared with the contralateral non-ischemic kidney, the internal control. This indicates that systemically administered stem cells migrated specifically to the injured tissue. However, no increase of injected HSC could be observed in injured or contralateral kidneys injected with SDF-1alpha. Moreover, neutralizing HSC-associated CXCR4 or endogenous SDF-1alpha did not result in a decrease of HSC in the kidney. It has been shown by several authors that HSC migrate towards SDF-1alpha in various experimental injury models. Our findings are not in accordance with this. To investigate whether a more severe and systemic type of danger was required for SDF-1alpha-dependent HSC migration, we performed additional experiments with a bilateral IRI model. Again, we could not observe an effect of the locally injected SDF-1alpha on the migration of HSC since both kidneys were engrafted with the same amount of exogenous administered HSC 1 day after IRI.

### **Conclusion**

In conclusion, systemically administered HSC successfully engraft ischemic injured renal tissue. The exogenous HSC had a preferred migration towards the ischemic injured kidney compared with the contralateral non-ischemic kidney. Surprisingly, local administration of SDF-1alpha or blocking the SDF-1alpha/CXCR4-axis did not result in an altered migration of HSC to ischemic injured kidney. In addition, no effect of the locally administered SDF-1alpha on the migration of exogenous HSC could be observed during bilateral IRI. Our results indicate that, in sharp contrast to other injured organs, migration of HSC to the ischemic damaged kidney occurs independently of the SDF/CXCR4 signaling axis.

## **O6. Depletion of NO availability during experimental chronic kidney disease causes permanent cardiac dysfunction**

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### **Background**

Reduced nitric oxide (NO) availability is implicated in the pathogenesis of cardiac damage in chronic kidney disease (CKD). Different animal models of CKD, however, do not consistently show cardiac dysfunction. We studied whether depleting NO reserves in a rat model of CKD affects cardiac function.

### **Methods**

Rats received either low-dose NO synthase inhibition with N( $\omega$ )-nitro-L-arginine (LNNA; 20 mg/L drinking water) or normal water from 3wk before to 8wk after subtotal nephrectomy (SNX) or sham-operation. This created 4 groups: SNX+LNNA, SNX, LNNA, and CON. Renal and cardiac function was measured regularly up to 15 wk. Data is shown as mean $\pm$ SEM.

### **Results**

Subtotal nephrectomy caused stable uremia from 4wk, which was mildly aggravated in SNX+LNNA at 8wk with plasma urea levels of 19.0 $\pm$ 1.5 mmol/L, compared to 13.8 $\pm$ 1.5 in SNX, 6.1 $\pm$ 0.1 in LNNA and 6.3 $\pm$ 0.3 mmol/L in CON ( $p < 0.01$ ).

Urinary NO metabolite excretion at 8wk was mildly reduced in SNX (0.9 $\pm$ 0.1  $\mu$ mol/24h/100g BW;  $p = 0.05$ ) and LNNA (0.8 $\pm$ 0.2  $\mu$ mol/24h/100g BW;  $p = 0.06$ ) compared to CON (1.2 $\pm$ 0.1  $\mu$ mol/24h/100g BW), but markedly depressed in SNX+LNNA (0.35 $\pm$ 0.03  $\mu$ mol/24h/100g BW;  $p < 0.05$  vs. all groups). Left ventricular (LV) hypertrophy occurred in both SNX and SNX+LNNA. At 4wk, when uremia was similar to SNX, LV fractional area change (LV-FAC) was severely decreased in SNX+LNNA (29 $\pm$ 1%) compared to SNX and CON (56 $\pm$ 3% and 52 $\pm$ 2%, resp.;  $p < 0.001$ ) and LNNA (40 $\pm$ 3%;  $p < 0.05$ ). The same differences in cardiac function were also apparent at wk8. Patchy areas of myocardial infarction and scarring were noted in SNX+LNNA ( $n = 6$ ), but not in the other groups.

Although LV-FAC was also slightly reduced in the LNNA-group at 4wk and 8wk ( $p < 0.05$  vs. SNX and CON), it returned to control levels after stopping LNNA (52 $\pm$ 2% vs. 51 $\pm$ 3% in CON and 46 $\pm$ 5% in SNX; N.S.,  $t = 11$ wk). Cardiac function remained compromised in SNX+LNNA at 11wk (31 $\pm$ 2%,  $p < 0.001$  vs all groups), and this was maintained up to 15wk (30 $\pm$ 2% vs. 44 $\pm$ 2% in SNX, 46 $\pm$ 1% in LNNA and 51 $\pm$ 3% in CON;  $p < 0.01$ .)

### **Conclusion**

Mild inhibition of NO synthase in SNX leads to markedly reduced urinary NO excretion as well as severely impaired LV function, both appearing to be partly independent from the decline in renal

function. Moreover, the LV dysfunction is permanent, perhaps due to structural damage. These data indicate that depletion of NO reserves is critical in causing cardiac disease in CKD.

#### **O7. Long-term adaptation of renal function is preserved after kidney donation in older donors**

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##### **Background**

Over the past decades, age of living kidney donors increased, reflecting the need to expand the donor pool. In our center, mean donor age increased from 40 years in the 80s to 50+ nowadays. Previously, we found older donors to be at increased risk for post-donation renal function impairment (CKD stage III). Also, post-donation renal reserve capacity was lower in older donors. It is unknown whether these findings compromise long-term donor renal function.

##### **Methods**

In our center, GFR (iothalamate), effective renal plasma flow (ERPF, hippuran) and filtration fraction (FF;  $GFR/ERPF \times 100$ ) are measured 4 months before and 2 months after donation. All 41 donors (mean age at donation 45.10) who underwent donor nephrectomy in our center in 2000-2001 were invited for long-term GFR assessment. 29 donors consented (78%); there was no difference in age, BMI or short-term renal function in donors available vs. lost to follow-up. Mean follow-up was  $6.0 \pm 0.6$  years. Data were analyzed by break-up at median age at follow-up, obtaining two groups with a range of 27-53 and 53-76 years.

##### **Results**

GFR ( $ml/min/1.73m^2$ ) was significantly higher in younger donors vs. older donors both before ( $111 \pm 11$  vs.  $101 \pm 11$ ;  $p < 0.05$ ) and 2 months after donation ( $72 \pm 7$  vs.  $64 \pm 7$ ;  $p < 0.01$ ). Long-term GFR was also higher in younger donors ( $81 \pm 8$  vs.  $71 \pm 9$ ;  $p < 0.01$ ). However, an adaptive rise in GFR over 6 years' time was similarly present in both younger and older donors. In both age groups, FF was stable on follow-up, so the long-term rise in GFR reflected a rise in perfusion rather than glomerular hypertension. Urinary protein leakage was low and similar in both groups at 6 years ( $0.25$  vs.  $0.21$  g/day). Though there were no significant differences in blood pressure between the age groups before or short-term after donation, MAP was higher at 6 years in older donors ( $99 \pm 7$  vs.  $91 \pm 7$  mmHg,  $p < 0.01$ ).

##### **Conclusion**

In conclusion, not only in younger but also in older kidney donors, the long-term adaptive response to donor nephrectomy apparently overcomes age-related renal function decline. Our data support current practice of accepting older donors, but careful screening and monitoring remains warranted.

#### **O8. Myocardial perfusion falls during hemodialysis**

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##### **Background**

Previous studies suggest that hemodialysis can elicit myocardial ischemia. Myocardial ischemia may be involved in the pathophysiology of dialysis hypotension and may contribute to the high cardiovascular event rate of dialysis patients. We studied whether myocardial hypoperfusion develops during hemodialysis and whether it leads to left ventricular (LV) dysfunction defined as the development of regional wall motion abnormalities (RWMA).

##### **Methods**

Gated  $^{13}N$ -NH<sub>3</sub> Positron Emission Tomography (PET) was used to quantify changes in myocardial perfusion, LV wall motion, cardiac output (CO), LV end-diastolic (LVEDV), and end-systolic volume (LVESV) in seven hemodialysis patients with an uneventful cardiac history and stable hemodialysis sessions. Three PET scans were performed: before dialysis and at 30 and 220 min of dialysis.

##### **Results**

At 30 min of dialysis without significant fluid removal, myocardial perfusion had fallen 13.5% ( $p < 0.05$ ) while CO, LVEDV and LVESV were 4.6%, 5.6%, and 6.9% lower, respectively. At 220 min of dialysis, after ultrafiltration of  $2.5 \pm 0.9$  liter, the average myocardial perfusion had fallen 26.6% ( $p < 0.05$ ) from baseline; CO, LVEDV, and LVESV, were 21.0%, 31.1% and 36.4% lower, respectively. New LV

RWMA developed at 220 min in two patients and were associated with relative perfusion defects in the corresponding regions.

#### **Conclusion**

Myocardial perfusion decreases during hemodialysis and myocardial hypoperfusion is associated with the development of systolic LV dysfunction. These changes occur in non-hypotension prone patients. As myocardial perfusion falls already early during hemodialysis without significant fluid removal, not only ultrafiltration-induced hypovolemia but also acute dialysis-mediated factors seem to play a role.

#### **O9. Complement activation by tubular cells is mediated by properdin binding**

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#### **Background**

Proteinuria is a strong and independent predictor of progression to end-stage renal disease in various forms of kidney disease. Activation of filtered complement products on the brush border of the tubular epithelium is thought to be a key factor underlying proteinuria-induced tubulointerstitial injury. However, the mechanism of complement activation on the proximal tubules has not yet been elucidated. We studied the role of properdin in complement activation on human renal proximal tubular epithelial cells (PTEC).

#### **Methods**

Immortalized HK-2 as well as primary PTEC lines were tested for binding of properdin. To determine the functional consequences of properdin binding, PTEC were incubated with properdin-depleted human serum after pre-incubation with properdin. Deposition of complement activation products on the cells was assessed by flow cytometry, excluding all dead cells from analysis. Human umbilical vein endothelial cells (HUVEC) were used as a control.

#### **Results**

Properdin binds dose-dependently to PTEC whereas no significant binding to HUVEC was detected. Addition of 20% normal human serum to PTEC resulted in complement activation with deposition of C3 and C5b-9 which was almost completely abolished when properdin-depleted serum was used. Pre-incubation of PTEC with properdin before addition of properdin-depleted serum fully restored complement deposition.

#### **Conclusion**

We conclude that properdin binding to PTEC acts as a focal point for alternative pathway activation and appears to be the rate limiting step in tubular complement activation. Interference with properdin binding to tubular cells may provide a target in the treatment of proteinuric renal disease.

#### **O10. Simultaneous assessment of Extracellular Volume and Glomerular Filtration Rate by the constant infusion method of 125I-iothalamate**

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#### **Background**

Disturbances in extracellular volume (ECV) are important in renal disease and its complications such as hypertension and left ventricular hypertrophy. However, ECV is seldom specifically addressed as its measurement is not part of the usual diagnostic armamentarium. The distribution volume (Vd) of filtration markers such as iothalamate and inulin typically equals ECV, so ECV could be estimated from data obtained during GFR measurement. This would also allow normalization of GFR to ECV that has been advocated as superior over normalization to BSA.

#### **Methods**

We studied the agreement between ECV assessed simultaneously as Vd of 125I-iothalamate (IOT) and Vd of bromide (50 mg/kg NaBr orally), in 50 subjects over a wide range of renal function (median 65, range 20-174 ml/min). VdIOT was measured during GFR measurement (steady state infusion of IOT). The reproducibility of VdIOT was tested in 26 volunteers, studied two times on different days during standardized Na<sup>+</sup> intake. Finally, in 150 potential kidney donors (63 men, 87 women) we measured VdIOT, GFR and assessed the impact of normalization to ECV versus BSA.

#### **Results**

VdIOT strongly correlated with Vdbromide ( $r=0.933$ ,  $p<0.01$ ), the Bland Altman plot showed deviations in the range of -2.6 to 3.1 L, without systematic error. Reproducibility assessment showed a mean

difference of 0.6% with a coefficient of variation of 9.3%. In n=150 GFR (130±28 vs 102±25 ml/min, p<0.01) and VdIOT(22.9±4.5 vs 17.3±2.5 l, p<0.01) were higher in men. GFR/BSA was also higher in men (105±20 vs 96±22 ml/min/1.73m<sup>2</sup>, p<0.01) but after normalization to ECV GFR was similar for men and women (5.8 ±1.1 vs 5.8 ±1.2 ml/min/l, ns).

#### **Conclusion**

Thus, VdIOT agrees well with Vdbromide, and has good reproducibility. So, ECV can be reliably assessed during measurement of true GFR. The latter are expensive and limited to specialized nephrology settings. Extending its yield optimizes the use of limited resources and allows to better document the role of volume expansion in the complications of renal disease. Finally, normalization of GFR by ECV may have implications for the interpretation of alleged sex-differences in renal function.

#### **O11. A Novel high efficient bio-assay to monitor microbiological purity of dialysis fluids.**

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#### **Background**

Microbial contamination of the dialysis fluid has a pro-inflammatory potential. The increased application of high-flux hemodialysis and on-line hemodiafiltration has made the need for ultrapure dialysis water even more stringent. Classical test methods for bacteriological contamination, such as bacteriological cultures or the Limulus Amebocyte Lysate (LAL)-test, fail to detect a substantial number of contaminants with intact lipopolysaccharide (LPS) as the only exception.

#### **Methods**

We developed a novel bio-assay for dialysis water contaminants using a monocytic THP-1 cell line. After a 24h rest period, calcitriol (10nM)-differentiated (72h) THP-1 cells, were incubated overnight (24h), in the presence of dialysis fluid samples (1/1) or samples containing potential activating microbiological agents. Secretion of IL-1β (pg/ml) was detected in the cell culture supernatant as a parameter of biological activity of the dialysis fluid. To validate the sensitivity of this test method to various types of contaminants, response to peptidoglycan (PGN), short bacterial DNA fragments and LPS fragments was compared to the response observed with the LAL-test.

#### **Results**

The presence of peptidoglycan (0.1; 1; 5; 10; 50; 100; 500; 1000; 5000 ng/ml) induced IL-1β secretion from 5 ng/ml on (P<0.05) whereas the classical LAL-test remained unresponsive. Likewise, addition of short bacterial DNA fragments (2006 stimu; 1μM and K3; 10μM) caused a significant IL-1β induction but no LAL-response. LPS fragments (MW<5kD) from *P. aeruginosa* induced no LAL-response, per se, but showed a marked biological activity. Intact LPS induced a significant IL-1β secretion versus control in a dose dependent manner from a concentration of 0.01 ng/ml on. A comparable evaluation with a biological test based on whole blood emanated in more scattered and/or less sensitive results.

#### **Conclusion**

The present data show that this novel bio-assay detects bacteriological derivatives which cannot be found by the classical screening methods. Application of this assay is useful to reveal contaminants which otherwise go undetected aiming at the timely prevention of biofilm formation in the dialysis circuit and micro-inflammation in the hemodialysis patient.

#### **O12. Histological examination of kidneys from old non-heart-beating donors before implantation**

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#### **Background**

Transplantation of kidneys from older donors expands the donor pool but is associated with inferior graft survival. Kidneys that are not expected to result in acceptable graft function may be used as dual transplants to further increase the number of kidney transplantations. Although several algorithms for selection of kidneys from older donors have been suggested in the literature, the relative merits of these strategies has not been studied systematically. Therefore, we retrospectively evaluated diagnostic accuracy and reproducibility of three selection algorithms in an unselected series of kidneys from marginal donors.

## Methods

From 1994 to 2005, 199 non-heart-beating donor kidneys were transplanted at our institution of which 52 kidneys were procured from donors 60 years or older. Pre-implantation biopsies of older kidneys (N=49) were retrospectively assessed by three independent pathologists with experience in nephropathology (C.J.P., R.J.v.S. and R.G.). Biopsies were scored for glomerulosclerosis, interstitial fibrosis, tubular atrophy and arterial narrowing (Remuzzi et al, NEJM, 2006). Functional kidney weight was calculated as kidney weight multiplied by the fraction of non-sclerosed glomeruli. Finally, donor GFR was estimated by the abbreviated MDRD formula.

## Results

Kidneys from older non-heart-beating donors had significantly worse graft survival than kidneys from younger donors (70 vs. 53% after 5 years,  $P=0.01$ ). Within the series of older donors, donor GFR ranged from 48 to 134 mL/min and correlated with functional kidney weight but not with donor age or Remuzzi score. Donor age and donor GFR did not predict kidney function at 1 year after transplantation nor graft survival censored for graft loss due to complications in the immediate post-operative period. Functional kidney weight was highly correlated to kidney function at 1 year after transplantation ( $R=0.58$ ,  $P<0.05$ ), but did not predict graft survival. In contrast, Remuzzi score was correlated to both GFR after 1 year ( $R=0.41$ ,  $P<0.05$ ) and graft survival (HR 1.25,  $P<0.05$ ) for all observers. However, predictive value of the Remuzzi score of two observers was lost when kidneys were divided into categories of mild, moderate and severe chronic injury as suggested by Remuzzi and colleagues. Diagnostic accuracy was regained when kidneys were divided according to optimal cut-off points for the individual observers. Interobserver agreement for Remuzzi categories (mild, moderate and severe chronic injury) was fair ( $k=0.38$ ) with systematic bias in assessing tubular atrophy and interstitial fibrosis. Interobserver agreement differed considerably among the scored items, with best agreement on glomerulosclerosis ( $k=0.81$ ) and worst agreement on interstitial fibrosis ( $k=0.24$ ). Elimination of interstitial fibrosis from the Remuzzi score did not affect diagnostic accuracy (ROC AUC 0.71 vs. 0.71).

## Conclusion

Kidneys from older non-heart-beating donors have reduced graft survival compared to kidneys from younger donors. Biopsy examination according to the Remuzzi score predicts both function and survival of these kidneys. Contrarily, donor GFR and functional kidney weight are not associated with graft survival. Since the predictive value is lost due to systematic bias between observers when kidneys are divided into categories, the Remuzzi score may be improved by eliminating assessment of interstitial fibrosis and by applying individualized cut-off points. Nevertheless, histological examination of kidney biopsies before implantation provides valuable prognostic information that is useful for selection of marginal kidneys for transplantation.

## O13. Immunological monitoring of renal transplant recipients to predict acute allograft rejection following the discontinuation of tacrolimus.

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## Background

To avoid adverse events, transplant patients would benefit from reduction of immunosuppression providing that graft rejection is prevented. To realize this, in vitro monitoring tools are needed to identify patients in whom immunosuppression can be safely reduced. In this study, we have evaluated a number of immunological markers in blood of patients in whom tacrolimus was withdrawn at six months after renal transplantation.

## Methods

The alloreactive precursor frequency of CD4+ and CD8+ T cells, the IL-2 and IFN- $\gamma$  producing potential of T cells, the frequency of T cell subsets, and the functional capacity of CD4+CD25+FoxP3+ regulatory T cells (Treg) were analyzed before transplantation and before tacrolimus reduction. The results were compared between patients that experienced an acute rejection after tacrolimus withdrawal (rejectors;  $n=15$ ) and a matched control group of patients without acute rejection (non-rejectors;  $n=28$ ).

## Results

Rejectors and non-rejectors did not differ with respect to the alloreactive precursor frequency of CD4+ and CD8+ T cells and the IL-2 and IFN- $\gamma$  producing potential of T cells. However, prior to tacrolimus reduction, the ratio between memory CD8+ T cells and Treg was higher in rejectors compared to non-rejectors ( $P<0.01$ ). Rejectors also had a higher ratio between memory CD4+ T cells and Treg

( $P < 0.05$ ), and low ratios ( $< 20$ ) were only observed in non-rejectors. Moreover, between the time of transplantation and the start of tacrolimus withdrawal, rejectors showed in general an increase in the percentage of naïve T cells and a reciprocal decrease in the percentage of effector T cells, while in non-rejectors a diverse pattern of changes was observed. A low memory CD4+ T cell : Treg ratio or a decrease in the proportion of naïve T cells was present in 76% of the non-rejectors, while these markers were absent in all rejectors. The proportion of Treg within the CD4+ T cells decreased after transplantation, but anti-donor regulatory capacity of Treg remained unaltered in rejectors and non-rejectors.

### **Conclusion**

In conclusion, immunological monitoring revealed an association between acute rejection following the withdrawal of tacrolimus and 1) the ratio of memory T cells and Treg prior to the start of tacrolimus reduction, and 2) changes in the distribution of naïve, effector and memory T cells over time.

### **P1. Is actigraphy a valid alternative to PSG for sleep measures in hemodialysis patients?**

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### **Background**

Sleep problems are frequently seen in hemodialysis patients. These problems have a major effect on quality of life. Polysomnography (PSG) is still the golden standard for the diagnosis and quantification of sleep disorders. This method is objective, but expensive and invasive. Actigraphy has been suggested as an alternative to PSG in assessing certain sleep disorders. The purpose of this study was to investigate whether actigraphy can be used for the detection of sleep problems in hemodialysis patients, in stead of PSG.

### **Methods**

Actigraphy and PSG were used to identify sleep problems. To investigate if actigraphy is an alternative to PSG in these patients the following parameters were used: sleep onset latency, sleep efficiency, intermittent wake time percentage, actual sleep time and awake index. These sleep parameters of PSG and actigraphy were correlated by means of Pearson correlation analysis. In addition, specificity, sensitivity and accuracy were obtained from epoch-by-epoch comparison of PSG and actigraphy.

### **Results**

Sleep problems were frequently seen in these hemodialysis patients ( $n = 15$ , 4 women, 11 men). Sleep efficiency, awake index and intermittent wake time percentage were impaired. There was a significant correlation between PSG and actigraphy for sleep onset latency ( $r = 0.60$ ,  $p < 0.05$ ), percentage actual awake time ( $r = 0.83$ ,  $p < 0.01$ ), sleep efficiency ( $r = 0.77$ ,  $p < 0.01$ ) and awake index ( $r = 0.83$ ,  $p < 0.01$ ). A trend in correlation was found for actual sleep time ( $r = 0.54$ ,  $p = 0.07$ ). Sensitivity, specificity and accuracy were respectively 0.95, 0.70 and 0.83.

### **Conclusion**

Sleep parameters obtained by actigraphy are strongly correlated to the sleep parameters measured with the standard method of PSG. Therefore, when sleep questionnaires indicate the presence of sleep disturbances, actigraphy can be used to objectify sleep disorders. However, when specific questions about sleep architecture are warranted, PSG is still the golden standard. If this is not the case, actigraphy is a valid alternative for PSG in sleep measures of hemodialysis patients.

### **P2. Influence of the preceding dwell time on the peritoneal equilibration test with 3.86% glucose in automated peritoneal dialysis**

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### **Background**

Recently it has been advised to perform the standardized peritoneal equilibration test (PET) with 3.86% glucose instead of 2.27% glucose solution. This would provide better information on ultrafiltration (UF) and is more sensitive to detect clinically significant UF failure. Point of concern has been the influence of the preceding dwell on the 3.86% glucose PET results. When most patients were on CAPD, the prior exchange has been approximately 8 hours, but with the growing number of patients treated with some form of APD this is no longer the case. Aim of this study is to compare net

UF, small solute transport, sodium sieving and transport type in APD between a PET preceded by a long dwell and a PET preceded by short dwells.

#### **Methods**

In 10 APD patients we performed two 3.86% glucose PETs of 4 hours in random order (A and B). PET A was preceded by a long day-dwell (>8 hours) using 3.86% glucose the day before and a nightly APD scheme with short dwells (< 3 hours) (standardized). PET B was performed after a long night dwell of > 8 hours using a 3.86% glucose solution. For this PET the patient did not perform the nightly APD scheme with short dwells as usually. Serum and dialysate samples were taken before instillation, after 1 and 4 hours. UF volumes and D/P ratios were compared for PET A and B.

#### **Results**

The tests were done within a mean period of 8 days (range 5-12 days). Mean total UF of PET A and B was  $671 \pm 251$  mL and  $612 \pm 189$  mL, respectively (N.S.). D/P creatinine after 4 hours was  $0.67 \pm 0.08$  in PET A and  $0.71 \pm 0.07$  in PET B ( $p=0.002$ ). D/P urea after 4 hours in PET A was  $0.89 \pm 0.04$  and in PET B  $0.90 \pm 0.05$  (NS). After 4 hours the Dt/D0 glucose were identical,  $0.31 \pm 0.05$  in PET A and  $0.31 \pm 0.05$  in PET B. Dip D/P sodium after 1 hour (sodium sieving) in PET A was  $0.883 \pm 0.023$  and in PET B  $0.889 \pm 0.016$  (NS). Classification of transport categories was identical for 8 out of 10 patients.

#### **Conclusion**

In this study no influence was found of the preceding dwell time on net ultrafiltration or free water transport, estimated using the maximum dip in D/P sodium in the first phase of the dwell, in a PET performed with a 3.86% glucose solution. Also the characterization into a transport type was not influenced significantly. The longer dwell time did increase the D/P ratio of creatinine. This can be explained by a higher content of the solute in the residual volume, owing to the longer equilibration time. Applying a rinsing procedure prior to the PET can possibly avoid this.

### **P3. A new and cost-effective strategy to implement the K-DOQI guidelines on CKD-MBD at the hemodialysis unit**

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#### **Background**

Disturbances in bone/mineral metabolism (CKD-MBD) are common in hemodialysis patients and accelerate the development of cardiovascular calcifications leading to increased mortality. Calcium can effectively lower phosphorus levels and decrease PTH, but its clinical utility is limited by inadequate use and the risk of vascular calcifications. Paricalcitol and mimpara can effectively lower PTH, but can increase costs. Use of an algorithm in combination with education of patients can address these issues. Our objective was to increase the number of patients within K-DOQI targets and decrease calcium load to a maximum of 2 gram/day without increased use of paricalcitol or calcimimetics.

#### **Methods**

An algorithm to guide changes in prescription of medication was introduced in January 2007. A Ca/P working group consisting of a medical doctor and 5 nurses was started to implement this algorithm and educate patients. The percentage of patients on chronic hemodialysis (>90 days) whose Ca, P and PTH were within K-DOQI targets were assessed from October 2006 to July 2007 ( $n=31$  patients). We also assessed the use of P-binders and active vitamin D, as well as the use of calcimimetics during this period.

#### **Results**

In July 2007 an improvement for all K-DOQI targets was observed as compared with the mean values of October 2006 and January 2007: The percentage of patients with a Ca level between 2,1 and 2,37 mmol/l increased from 45% to 59%, with a P level between 1,13 and 1,78 mmol/l from 45 to 56%, with a  $Ca \cdot P < 4,4$  mmol<sup>2</sup>/l<sup>2</sup> from 57% to 71% and with PTH between 15,8 and 31,6 pmol/l from 41 to 45%. In the same period, the Ca-load in P-binders was decreased from 1,5 to 1,0 gram a day. Non-calcium-containing P-binders were only moderately increased (lanthanumcarbonate from 0,9 to 1,2 tablets/day, sevelamer from 6,2 to 6,4 tablets/day). The prescription of alphacalcidol increased from 2,4 mcg/week to 3,9 mcg/week. The mean amount of paricalcitol decreased from 5,4 mcg/week to 4,7 mcg/week. The mean amount of calcimimetics remained unchanged (2,3 mg/dg).

#### **Conclusion**

An cost-effective strategy was found to reach K-DOQI targets and decrease calcium-load at the hemodialysis unit.

**P4. Design of the ICD2 study: A prospective randomized controlled trial to evaluate the prevention of sudden cardiac death using implantable cardioverter defibrillators in dialysis patients.**

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**Background**

Dialysis patients have an extraordinary high risk of death. Sudden cardiac death (SCD) is the single largest cause of death in dialysis patients. Implantable Cardioverter Defibrillator (ICD) therapy has demonstrated to reduce the risk of SCD and all cause mortality in high risk patients. Whether ICD therapy benefits dialysis patients is unknown. The main objective of this study is to assess whether ICD therapy in dialysis patients aged 55-80 years can reduce sudden cardiac (arrhythmic) death.

**Methods**

In order to calculate the number of patients needed to perform this study, and identify device-related risks we reviewed a US database ([www.usrds.org](http://www.usrds.org)), a dutch registry ([www.reninc.nl](http://www.reninc.nl)) and the literature.

**Results**

Age is the best predictor of all cause mortality and SCD in dialysis patients. Mortality rates rise from 50 deaths/1000 patients years for dialysis patients younger than 45 years to 300 deaths/1000 patients years for patients older than 65 years. SCD accounts for approximately 30% of all cause mortality in all age groups. In the Netherlands the annual mortality rate was 30% for all patients aged 65 to 74 between 1995-2002, with a slight decrease in the two years thereafter. A further decrease in all cause mortality due to more intensive surveillance during the study and possibly to new treatment modalities is likely. Therefore we estimate the annual rate of all cause mortality in patients aged 55-80 years at least 22%. Assuming that patients enter the study during an accrual period of two years and a follow-up of three years, we estimate a SCD rate of 19% in the standard therapy group and a 6% rate in the ICD group. A two-sided log rank test with an overall sample size of 200 subjects achieves 90% power at a 0.05 significance level to detect a difference of 13% between the two groups (the proportions of patients surviving 3 years).

Several device-related risks are known: (small) pneumothorax (1.5-3%); pocket hematoma (3%); ICD infection (3-6%); dialysis shunt occlusion (% unknown) and inappropriate shocks (5-6%).

**Conclusion**

It is feasible to perform a study to assess whether ICD2 therapy can reduce SCD in dialysis patients: The possible benefits (reduction of SCD, cardiovascular check-up) outweigh the risks. A prospective randomized open-label trial, the ICD2 study, has started in april 2007: 200 patients will be randomised to either ICD therapy (ICD group), or no ICD therapy (control group).

**P5. Anemia is an independent predictor of death-censored graft loss in kidney transplant recipients**

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**Background**

Post transplant anemia (PTA) is common after renal transplantation (RTx). Recent data suggested an effect on death censored graft loss, but did not establish whether this is an independent effect.

We analysed the prognostic impact of PTA (hematocrit<0.36 at 1 year after RTx) in a well-documented single center cohort.

**Methods**

Data on 830 RTx performed in our center between 1984 and 2002 were analysed for determinants of allograft loss and mortality by regression analysis.

**Results**

At 1 year after Tx 15.2% of the patients had PTA. Distribution of hematocrit levels remained stable throughout follow-up. During follow-up, total graft loss amounted to 31.9%, death censored graft loss to 11.8%, with a mortality of 20.1%. Median time to graft loss was 7.7 years and 8.1 years to death. PTA was a predictor of death censored allograft loss ( $p<0.001$ , log rank), overall graft loss and mortality. On Cox-regression analysis, death censored allograft loss was predicted independently by PTA (HR, 13.38;  $p=0.003$ ), patient's gender, age at transplantation, body mass index, and glomerular

filtration rate (GFR). Donor's gender, ischemia times, HLA mismatches, mean arterial pressure, RAAS blockade medication, re-transplantation, glycemia, CMV infection, and modality of dialysis did not contribute significantly. A significant interaction was present between PTA and GFR, with a pronounced effect of PTA on death censored graft loss in patients with low, but not high GFR.

#### **Conclusion**

We conclude that PTA is an independent predictor of allograft loss, in particular in patients with GFR<60 ml/min/1.73 m<sup>2</sup>, as well as of mortality.

#### **P6. Kidney transplant patients with ATN have low numbers of circulating CD34+ cells compared to complication-free patients**

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#### **Background**

During kidney transplantation, the graft is exposed to ischemia/reperfusion injury. This results in impaired vascularization and/or vascular lesions in the transplant and can cause ischemia-induced acute tubular necrosis (ATN). Moreover, a history of cardiovascular disease (CVD) is a risk factor for ATN (in both the donor and the recipient). Endothelial progenitor cells (EPCs) mediate vascular repair and could repair vascular damage after ATN. However, cardiovascular problems are known to be inversely correlated with circulating EPC numbers. In this study we assessed numbers of EPCs in kidney transplant recipients undergoing ATN, since this issue has not been previously addressed.

#### **Methods**

In a cross-sectional study, eleven consecutive kidney transplant recipients were included  $\pm$  14 days after TX with biopsy proven ATN. Twenty-two consecutive patients with stage 4 chronic renal failure (CKD; mean duration of stage 4 chronic renal failure according to the KDOQI guidelines, 57 $\pm$ 4 months, range 9-168 months) were included as controls. Twenty-two healthy individuals were also included. Mononuclear cells (MNC) were isolated from 10 ml of heparinized peripheral blood by lymphoprep density gradient centrifugation. Circulating CD34-expressing EPCs were quantified by flow cytometry.

#### **Results**

In ATN patients, numbers of circulating EPCs at short term after kidney transplantation were drastically decreased (mean 378 CD34+ EPC/ml blood, range 94-1100 cells/ml). This number was significantly lower than in complication-free transplantation patients ( $p<0.05$ , mean 830 CD34+ EPC/ml blood, range 63-3102 cells/ml). In the complication-free group, circulating EPC numbers were significantly lower than in healthy controls ( $p<0.01$ ; mean 1370 CD34+ EPC/ml blood; range 800-2360 cells/ml).

To investigate the effect of the uremic state (as a result of ATN) on EPCs, circulating EPC numbers were compared with CKD patients. Numbers of CD34+ EPCs in CKD patients (mean 955 cells/ml blood, range 430-1780) were significantly lower than in healthy controls ( $p<0.05$ ). Healthy controls and CKD patients had both significantly more circulating EPC than ATN-developing kidney transplant recipients ( $p<0.001$  and  $p<0.05$ , respectively).

#### **Conclusion**

We found decreased numbers of EPCs after ATN compared to the complication-free patients after kidney transplantation. CKD patients, which had significantly lower EPC numbers than healthy controls, show the effect of the uremic state of the patients. As a result of ATN, wastes are not properly removed, leading to an accumulation in the circulation. This uremic intoxication is known to cause defects in cellular function, which might lead to decreased numbers of EPC. In addition, ATN patients more often have a history of CVD, which is inversely correlated with EPCs. More research is necessary to find out whether the uremic state destroys EPCs.

#### **P7. Differences in DNA methylation between rat strains after renal ischemia-reperfusion injury**

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#### **Background**

DNA methylation is a post-transcriptional regulatory mechanism. Addition of methyl group to CpG islands in the promoter region is catalyzed by DNA methyltransferase enzymes (DNMTs) and the

result is inhibition of gene transcription. It was shown by Endres et al. that in experimental brain ischemia there is less severe damage in presence of hypermethylation and it was partly dependent on DNMTs activity. Renal ischemia/reperfusion (I/R) injury is one of the leading causes of acute renal failure and allograft dysfunction. The role of DNA methylation in renal response to ischemia has not been studied yet. We investigated renal DNA methylation status and kidney tissue damage after I/R injury in different rat inbred strains.

#### **Methods**

Male BN/RijHsd (BN), MWF/ZtmHsd (MWF), SS/JrHsd (SS), and SHR/NHsd (SHR) rats (n=6) were obtained from Harlan Nederland B.V. At nine weeks of age rats were subjected to unilateral I/R for 45 minutes. At 24 hours after reperfusion rats were sacrificed. Parts of kidney tissue for DNA and RNA isolation were placed in liquid nitrogen. DNA was isolated from both the ischemic and the untreated kidney, and global methylation was determined using the Luminometric methylation assay (LUMA). RNA was isolated to determine gene expression of damage markers (IL1B, MCP1 and KIM1) and DNA methylation enzymes (DNMT1, DNMT3A, and DNMT3B). For quantitation of mRNA levels, the ABI Prism 7900 HT sequence detection system (Applied Biosystems) was used with Sybr green chemistry (Abgene). To determine the amount of renal structural damage, immunohistochemistry for HO-1 and ED-1 was performed on paraffin sections.

#### **Results**

Significant differences in effect of ischemia/reperfusion on DNA methylation levels between the four strains were found in ischemic kidneys. Increased methylation (hypermethylation) after an ischemic injury was present in kidneys of BN and SS strains ( $P=0.002$  and  $P=0.025$ , respectively) as compared to non-treated, contralateral kidney. In the two other investigated strains (MWF and SHR) the amount of global DNA methylation in ischemic kidney did not differ from basal methylation presented by the control kidney (normomethylated kidneys). Furthermore, mRNA expression of damage markers (IL1B and MCP1) after renal I/R, was significantly increased only in the two normomethylated strains (MWF:  $P=0.003$  and  $P=0.037$  respectively and SHR:  $P=0.002$  and  $P=0.005$  respectively). Also, these two strains showed a two-fold higher KIM1 expression compared to the two hypermethylated strains (BN and SS). Thus, in strains with hypermethylation after I/R, expression of damage markers was attenuated. This only confirms the key concept of DNA methylation as a regulatory system that controls gene transcription. In regard to kidney tissue damage, there was a significant increase of HO-1, an oxidative stress marker, after the I/R in all four strains. When looking at the relative amount of the HO-1 in the tissue of the injured kidneys, it followed the pattern of DNA methylation levels, with more presence in normomethylated strains. Also, there was an increase of macrophage influx upon injury measured by ED-1 in SHR ( $P=0.001$ ), SS ( $P=0.001$ ) and MWF ( $P=0.001$ ). Renal expression of DNMT1, DNMT3A and DNMT3B was not significantly different between strains.

#### **Conclusion**

Strain comparison reveals a reciprocal effect of I/R on DNA methylation status and renal damage markers. This suggests that increased DNA methylation levels play a role in the renal response to ischemia reperfusion, attenuating the severity of damage. Recognizing it as a factor that contributes to tissue outcome is a step forward to understanding the complexity of renal response to ischemic injury. In our study the strain-dependent difference in DNA methylation could not be explained by different expression of DNA methyltransferases DNMT1, DNMT3A and DNMT3B. Further exploration of the role of DNA methylation after I/R may have the potential to uncover endogenous protective mechanisms.

#### **P8. Can mass screening for albuminuria identify subjects at increased risk of need for renal replacement therapy?**

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#### **Background**

Efforts should be made to detect renal disease at an early stage for timely start of renoprotective therapy to prevent the need for chronic renal replacement therapy (RRT). We investigated whether screening for urinary albumin concentration (UAC) in the general population identifies patients at increased risk of need for RRT.

#### **Methods**

In 1997 all 85,421 inhabitants of Groningen, the Netherlands, aged 28-75 yr were invited to collect a urine sample for UAC screening (nephelometry) and to fill out a questionnaire. 40,856 subjects

responded. Linkage of the n=85,421 cohort with the national RRT database (RENINE) identified 105 subjects that started RRT between screening and 31-12-2006 (9 yr of follow up). Hospital charts of these subjects were lifted to obtain clinical characteristics.

### **Results**

Characteristics of the 105 RRT subjects at time of screening: age 58 yr, female 53%, known with renal function impairment at their general practitioner 57%, treated with ACEi/ARB 30%. At screening 63% had a history of hypertension, 19% cardiovascular disease, 25% DM and 73% age>50 yr, whereas only 12% belonged to none of these ESRD risk groups. Subjects that started RRT and did not have UAC measured in the 1997 screening (n=58) had similar characteristics as RRT subjects that had UAC assessed (n=47). Of the 40,856 screening participants 92% was normoalbuminuric (UAC<20 mg/L), 7.1% microalbuminuric (20-200 mg/L) and 0.7% macroalbuminuric (>200 mg/L). In the group that started RRT these figures were 43%, 23% and 34%, resp. Odds ratios for micro- and macroalbuminuric subjects to start RRT were 7.1 and 113 resp. (both p<0.001) compared to normoalbuminuric subjects. Screening for UAC>20 mg/L identified 57% of the subjects that started RRT, of which 37% were not known with renal function impairment and 52% not treated with ACEi/ARB.

### **Conclusion**

In conclusion, screening for micro- and macroalbuminuria can be used to identify subjects at risk for need for RRT. However, the number of patients reaching this endpoint is small. Limiting mass screening for albuminuria to ESRD risk groups, such as those >50yr, will make screening more efficient.

## **P9. High protein intake in the general population is associated with increased risk for cardiovascular events, but not with a decline in renal function.**

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### **Background**

In western societies dietary pattern has changed towards higher protein intake (PI). Randomized trials have shown that patients with renal disease and a high PI have a more rapid renal function decline than patients with a protein restricted diet. These trials were underpowered to study cardiovascular (CV) events. The aim of this study was to investigate whether PI in the general population is associated with rate of renal function decline and risk for development of CV events and total mortality.

### **Methods**

Data were used of 8,483 subjects without renal disease (F: 49.9%, age: 49.8 yr) who completed three subsequent screenings (6.2 yr follow-up) in a prospective community based cohort study (PREVEND). Baseline daily PI was calculated from 24hr urinary urea excretion (Maroni formula).

### **Results**

Average PI intake was 1.19±0.27 g/kg/d. Our data showed no association between baseline PI and rate of renal function decline during follow-up (slope through 3 eGFR values, calculated with the MDRD formula), neither univariate (p= 0.6), nor after correction for age/gender (p=0.6), or for age/gender/ Framingham CV risk factors (p=0.3). In contrast, in all such models PI was significantly associated with CV event rate during follow-up (p<0.05). The associations appeared U-shaped, with highest event rates in subjects with high PI (HR: 1.3) and low PI (HR:1.2) compared to subjects with intermediate PI (reference). In contrast, total mortality and non-CV mortality showed a significant negative association with PI, with subjects with low PI showing an increased non-CV and total mortality rate (HR: 1.6, 1.4). We therefore hypothesize that the increased risk associated with low PI, is the consequence of co-morbidity with a poor nutritional status, and not of low PI per se.

### **Conclusion**

In conclusion, in the general population high PI does not predispose for rapid renal function decline but is associated with an increased propensity for CV events. Lowering protein intake may therefore be beneficial in subjects with a high PI.

## **P10. Baseline albuminuria predicts the efficacy of antihypertensives in prevention of cardiovascular events**

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## Background

Albuminuria has been proven to be associated with cardiovascular (CV) events in specific patient populations, but also in the general population. Based on suggestions in literature, we aimed to investigate whether the effect of antihypertensives in preventing CV outcome depends on baseline urinary albumin excretion (UAE) and whether there is an association with the type of antihypertensive drug.

## Methods

Data were used from the observational, community based Prevention of Renal and Vascular Endstage Disease (PREVEND) cohort study and pharmacy dispensing records (IADB.nl). Included were subjects with hypertension (SBP  $\geq 140$  or DBP  $\geq 90$  mmHg), no CV history, and no previous use of antihypertensives. UAE was assessed in two 24hr urines. CV outcome (following MACE definition) was obtained from Dutch hospital discharge and mortality registries.

## Results

During a mean follow-up of  $7.1 \pm 1.6$  years, 122 CV events were observed in the 1,185 subjects included. Start of antihypertensives as compared to non-use was associated with absolute risk reductions for CV events of 0.7% ( $p=NS$ ), 6% ( $p=0.08$ ), and 12.6% ( $p<0.05$ ) for all subjects, those subjects with  $UAE \geq 15$  mg/day ( $n=574$ ), and  $UAE \geq 30$  mg/day ( $n=323$ ), respectively. Cox regression analysis showed that the relative risk for CV events of those starting antihypertensive drug use versus non-use significantly depends ( $p<0.05$ ) on baseline UAE; with hazard ratios (HRs) of 0.87 ([95% CI,  $p$ -value] 0.61-1.26,  $p=NS$ ), 0.87 (0.48-1.60,  $p=NS$ ), 0.58 (0.36-0.94,  $p<0.05$ ), and 0.37 (0.20-0.68,  $p<0.05$ ), for all subjects and those subjects with  $UAE < 15$ ,  $\geq 15$  and  $\geq 30$  mg/day, respectively. These results were essentially similar after adjustment for age, sex, baseline characteristics, propensity scores and level of exposure to antihypertensive agents. Renin-Angiotensin-System (RAS) intervening treatment showed an adjusted HR of 0.51 (0.26-1.03,  $p=0.06$ ) for CV events if compared with non-RAS intervening treatment. Furthermore, high exposure to antihypertensive agents ( $\geq 0.75$  Defined Daily Doses (DDD)/day) was associated with a lower chance to reach the composite CV endpoint as compared to low exposure ( $< 0.75$  DDD/day) with an adjusted HR of 0.65 (0.39-1.08,  $p=0.10$ ).

## Conclusion

In conclusion, these data show an UAE dependent effect of antihypertensives on CV events in hypertensive subjects, with RAS intervening agents appearing to be more favorable than other non-RAS intervening agents. Since this study is observational, our results should be interpreted with caution. However, these data could serve as a rationale for an albuminuria-based 'screen-and-treat' program for hypertension in the prevention of CV events.

## P11. Dendritic Cells in ANCA-associated Vasculitis

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## Background

Dendritic cells have an important role in maintaining immune tolerance, and initiation of immune responses. Their involvement in ANCA-associated vasculitis (AAV) is unknown. In this study, the participation of dendritic cell subsets is investigated in AAV. For this purpose, renal biopsies from patients were analysed by immunohistochemistry.

## Methods

25 patients with biopsy-proven ANCA-vasculitis and 5 healthy controls (HC) with normal renal histology were included. Renal biopsies were stained for mature (CD208), immature (CD209), plasmacytoid (CD303) and Langerhans (CD1a) dendritic cell subsets. Furthermore, a T-cell staining (CD3) was performed. The interstitial cellular infiltrate was graded semiquantitatively from 0+ (=absence of cells) to 3+ (=numerous cells). An absolute count was performed for positive cells within the glomeruli.

## Results

Patients' biopsies contained an average of  $12 \pm 4$  glomeruli, whereas  $11 \pm 3$  glomeruli were found in HC. CD208+ and CD209+ cells were found within patients' glomeruli but not in HC ( $1 \pm 0.3$  vs.  $0.08 \pm 0.1$  cells/glom;  $2 \pm 0.3$  vs.  $0.1 \pm 0.07$  cells/glom). An average of  $0.3 \pm 0.1$  cell/glom expressed CD3 in patients while these cells were less often found in HC ( $0.1 \pm 0.7$  cell/glom).

Focal interstitial cellular infiltrates were observed in patients' biopsies but not in HC. Interstitial infiltration with CD3+ and CD209+ cells was assessed at an average of 1+; but some glomeruli and tubuli were surrounded by numerous (3+) CD3+ and CD209+ cells forming clusters, whereas other glomeruli/tubuli were unaffected. Only single interstitial CD1a+, CD208+ and CD303+ cells were found in patients' specimen.

Serial sections revealed that CD209+ cells were present in CD3+ rich areas. Areas exclusively consisting of CD209+ cells were found in some biopsies.

#### **Conclusion**

Immature dendritic cells and T-cells are found in renal biopsies from patients with AAV. Dendritic cells form aggregates in T-cell areas suggesting an interaction between these cell types, e.g. lymphoid neogenesis.

### **P12. The renal transcriptome in spontaneously hypertensive rats from birth to old age: the search for transcription factor candidates for each phase of development**

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#### **Background**

We hypothesized that renal transcriptomes would gradually change in SHR during phases of nephrogenesis (2d), prehypertension (2w), hypertension (24w) and old age (48w), and that by studying potential binding sites for transcription factors (TF) in phase-specific renal transcriptomes of SHR, TF could be recognized of potential relevance for the phenotype of each phase.

#### **Methods**

SHR was compared to age-matched WKY. Renal cortex of 5-8 rats/strain/age was subjected to oligonucleotide microarray (pooled samples) and quantitative PCR (qPCR; individual samples). Self-self comparison of SHR mRNA (6 arrays) enabled identification of unreliable genes. An absolute ratio of  $\log_2(\text{SHR}/\text{WKY}) > 0.7$  was considered significant (i.e. 3.4xS.D. of self-self comparison). Frequency of in silico predicted binding sites for TF in the 1K upstream region of regulated genes was compared with 200 genes centred around the mean ratio at each age using BIOMART, MATCH and proprietary software.

#### **Results**

Microarray revealed 123, 96, 187# and 501# regulated genes at 2d, 2w, 24w and 48w, respectively; #  $P < 0.01$  vs. other ages. Each age had a unique pattern (hierarchical clustering); 66 genes were modulated at two or more ages. Of these, 30% could not be linked to blood pressure (BP) by quantitative trait locus (QTL) at LOD score  $> 2.5$ , e.g. soluble epoxide hydrolase (Ephx2). Gene Ontology classification pointed at modulated ion transport at 2d, 2w and especially  $\text{Na}^+$  and  $\text{K}^+$  at 24w. Ephx2 expression was induced at all ages (qPCR,  $P < 0.01$ ); perinatal inhibition of Ephx2 in SHR persistently lowered systolic BP in the offspring. CTGF and Gstm1, both linked to BP QTL, were consistently induced and reduced in SHR, respectively (qPCR,  $P < 0.01$ ). Genes related to transcription were induced in SHR at 2d and 2w. In silico detection of TF binding sites of regulated genes revealed 4 TF binding sites to be significantly more frequent at 2d, 6 at 2w, 0 at 24w, 2 at 48w. S8 binding sites were frequent at 2d and 2wk. Two binding sites were less frequent in regulated genes: Elk-1 at 2d and 2w, and NRF-2 at 24w.

#### **Conclusion**

In SHR TF binding is most clearly associated with regulated genes during nephrogenesis. Although few genes were consistently induced or repressed in SHR, our study reveals new early and late candidate genes and potential transcription factors related to different phases of development of SHR.

### **P13. SIGIRR does not suppress TLR-mediated renal inflammation and dysfunction following early ischemia/reperfusion injury**

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#### **Background**

TLR's are expressed on leukocytes and renal cells and play a crucial role in the initiation of innate immunity upon ischemia/reperfusion (I/R) injury. Tight regulation of TLR signaling is therefore of crucial importance to dissolve excessive inflammation resulting in dampening of renal injury. One of the negative regulators of TLR signaling is the orphan receptor SIGIRR (single Ig IL-1R-related

molecule), which is predominantly expressed on epithelial tissues, particularly in the kidney. The functional relevance of this organ-specific expression however, remains to be elucidated. We therefore hypothesize that renal epithelium-associated SIGIRR is involved in regulating TLR-mediated innate immunity in the kidney upon renal I/R injury.

#### **Methods**

We used SIGIRR<sup>-/-</sup> and C57/Bl6J wild type mice (n=8/group) that were subjected to I/R injury by bilateral clamping of the renal arteries for 45 minutes followed by reperfusion. Mice were sacrificed after one day to determine renal functional parameters (plasma urea and creatinine levels), histopathology and renal inflammation. Sham-operated animals served as controls. In addition, we isolated primary tubular epithelial cells from wild type and SIGIRR<sup>-/-</sup> kidneys and subjected them to simulated ischemia by covering the cells with a monolayer of mineral oil for one hour, followed by medium-reperfusion. After one day, secreted cytokine/chemokine levels were determined in the supernatant by ELISA. Values are expressed as mean±SEM. Statistics were done using the independent samples T-test.

#### **Results**

One day after I/R injury SIGIRR<sup>-/-</sup> mice had a comparable degree of renal dysfunction compared with wild type mice as reflected by equal levels of plasma urea (52.35±1.91 vs. 52.80±1.49 mmol/l) and creatinine (171.00±11.43 vs. 170.00±7.76 micromol/l). Moreover, no difference was found in renal histopathology between SIGIRR<sup>-/-</sup> and wild type mice (4.00±0.12 vs. 4.28±0.13 arbitrary units/HPF, PAS-D stained sections). To evaluate the effect of SIGIRR deficiency on renal inflammation, we subsequently counted the number of interstitial neutrophils. Surprisingly, no difference was found between SIGIRR<sup>-/-</sup> and wild type mice one day after I/R injury (49.14±9.15 vs. 41.46±8.07 Ly6G+ cells/HPF). In addition, no difference was found in the secreted levels of keratinocyte chemoattractant (KC), interleukin-1beta (IL-1beta) and monocyte chemoattractant protein-1 (MCP-1) between SIGIRR<sup>-/-</sup> and wild type primary tubular epithelial cells when subjected to simulated ischemia.

#### **Conclusion**

These preliminary data suggest that SIGIRR is not important in the regulation of TLR-mediated inflammatory responses following early renal I/R injury in mice. Evidently, more research will be necessary to elucidate whether SIGIRR plays a role in dampening renal inflammation in later phases of I/R injury.