FREE COMMUNICATIONS

Experimental Chronic Kidney Disease

FC-20043

CASPASE INHIBITION REDUCES TUBULAR APOPTOSIS AND PROLIFERATION AND SLOWS DISEASE PROGRESSION IN POLYCYSTIC KIDNEY DISEASE (PKD)

YUNXIA TAO[1], JUN KIM, ROBERT W. SCHRIER AND CHARLES L. EDELSTEIN[1]
University of Colorado Health Sciences Center[1]

Increased apoptosis is a characteristic feature of polycystic kidney disease (PKD). The aim of the present study was to determine the effect of caspase inhibition on tubular cell apoptosis and proliferation, cyst formation and renal failure in the Han: SPRD rat model of PKD. Heterozygous (Cy/+) and littermate control (+/+) male rats at 3 weeks of age were treated with the caspase inhibitor, IDN8050 (10 mg/kg/d) via a minipump or vehicle for 5 weeks. On immunoblot analysis the active form of caspase-3 in kidney was significantly reduced in rats treated with IDN8050. The number of TUNEL positive tubular cells per cyst in the cortex was 0.3 in vehicle-treated Cy/+ and 0.01 in IDN8050 treated Cy/+ ($P < 0.05$). The 2 kidney/total body weight ratio was 0.9 in vehicle-treated +/+ ($n = 8$), 2.1 in vehicle-treated Cy/+ ($p < 0.001$ vs +/+) and 1.6 in IDN8050-treated Cy/+ ($P < 0.001$ vs Cy/+ $n = 6$). Cyst volume density (CVD)(%) was 0.4 in vehicle-treated +/+ ($n = 4$), 43 in vehicle-treated Cy/+ ($p < 0.001$ vs +/+) and 30 in IDN8050-treated Cy/+ ($P < 0.001$ vs Cy/+ $n = 6$). BUN was 23.5 in vehicle-treated +/+ ($n = 4$), 38 in vehicle-treated Cy/+ ($p < 0.001$ vs +/+) and 26 in IDN8050-treated Cy/+ ($P < 0.001$ vs Cy/+ $n = 4$). The number of PCNA positive cells per non-cystic tubule in the cortex were 0.04 in vehicle-treated +/+, 0.8 in vehicle-treated Cy/+ ($p < 0.001$ vs +/+) and 0.15 in IDN8050-treated Cy/+ ($P < 0.05$ vs Cy/+ $n = 4$), and the number of PCNA positive cells per cyst in the cortex was 1.0 in vehicle-treated Cy/+ rats and 0.12 in IDN8050 treated Cy/+ ($P < 0.05$, $n = 4$). In summary, in a rat model of PKD, caspase-inhibition with IDN-8050 1) decreases apoptosis and proliferation in cystic and non-cystic tubules; 2) inhibits renal enlargement by 44% and cyst volume density by 29%; and 3) attenuates the loss of kidney function.

FC-20044

VEGF SIGNALING, BUT NOT SMAD3 SIGNALING, ENGENDERS ALBUMINURIA IN DIABETIC NEPHROPATHY, POSSIBLY VIA ALTERATIONS IN NEPHRIN EXPRESSION

SHELDON CHEN[1], SUN HEE SUNG[1], NICHOLAS J. LAPING[2], FUAD N. ZIYADEH[1]
University of Pennsylvania Renal Division Philadelphia United States[1], Urogenital Biology, CVU CEDD, GlaxoSmithKline, King of Prussia, PA, USA[2]

The pathogenesis of albuminuria in diabetic nephropathy remains mysterious. Our earlier work did not demonstrate improvement of albuminuria by neutralizing TGF-β in type 2 diabetic db/db mice. Subsequently, neutralization of VEGF has successfully ameliorated albuminuria in rodent models of type 1 and type 2 diabetes. To explore the roles of TGF-β and VEGF without resorting to an antibody-based approach, Smad3-knockout mice were made diabetic with streptozotocin, and a pan-VEGF receptor inhibitor, SU5416, was administered to db/db mice.

Smad3-KO mice and their age-matched wildtype controls were randomized to receive vehicle or streptozotocin and remained diabetic for six weeks. Eight-week-old db/db and control db/m mice were injected intraperitoneally with vehicle or 2 mg/kg SU5416 twice-a-week for 8 weeks. In both experimental groups, the urine albumin excretion (UAE), measured by ELISA, increased with diabetes, rising ~3-fold in the STZ-wildtype mice and ~4-fold in the db/db mice. The increased UAE of diabetes was not improved in the diabetic Smad3-KO mice, but was significantly ameliorated in the db/db mice by SU5416 treatment. Based on structure-function studies, histologic parameters that correlate with albuminuria were measured. Diabetic GBM thickening was prevented in both Smad3-KO mice and SU5416-treated db/db mice. The mesangial matrix expansion of diabetes, quantified by electron microscopy, was prevented in Smad3-KO mice but not in SU5416-treated mice. Glomerular VEGF immunostaining, normally increased by diabetes, was unaffected by either Smad3-KO status or SU5416. Finally, nephrin immunofluorescence was diminished in db/db mice and was partially but significantly restored by SU5416.

Thus, overactivity of VEGF signaling, but not necessarily the Smad3 arm of TGF-β signaling, plays a central role in diabetic albuminuria. GBM thickening and mesangial matrix expansion can be dissociated from albuminuria, calling into question their pathogenetic roles in albuminuria. Lastly, the reduced nephrin levels of diabetic nephropathy could be provoked by maladaptive VEGF signaling.

FC-20045

REVERSAL OF HYDRONEPHROSIS IN THE MOUSE AS A MODEL FOR RENAL REPAIR AND REGENERATION

JOHN BERTRAM[1], ANITA COCHRANE[1], MICHELLE KETT[1], WARWICK ANDERSON[1], DAVID HUME[2], MELISSA LITTLE[2], SHARON RICARDO[1]
Monash University, Australia[1], University of Queensland[2]

The kidney has an inherent ability for recovery and regeneration following acute damage. The present study describes the structural, functional, and microarray analysis of the regenerative potential of the kidney following reversal of ureteral obstruction (R-UUO). Male C57 mice (20–25 g; $n = 6$/group/time point) underwent unilateral ureteral obstruction (UUO) for 10 days followed by R-UUO for 1, 2, 4, and 6 weeks. Ten days after UUO, mice demonstrated tubular atrophy, medullary ablation, and the development of severe interstitial fibrosis. UUO animals had a 38% fall in total GFR in comparison to sham-operated control animals. Following R-UUO at 2 and 6 weeks, two distinct areas were apparent in the renal parenchyma: one where tubules had repaired and displayed a normal histoarchitecture; and a second where the same kidneys demonstrated tubular and matrix remodeling. Elevated numbers of macrophages were evident in the focal area of remodeling in R-UUO kidneys compared to areas that had undergone repair ($2458.4 \pm 393.7/mm^2$ vs. $575.0 \pm 202.6/mm2$; $P < 0.01$). However, by 6 weeks there was a progressive decline in total numbers of macrophages in R-UUO kidneys compared to the obstructed kidneys of UUO mice. This was in co-occurrence with reduced type I, III, IV, and V collagen accumulation and resolving interstitial matrix expansion. At this time-point there was a significant but partial restoration of renal function as measured by GFR from individual ureteral catherization of the R-UUO kidneys in comparison to sham-operated control kidneys. In addition, we have used expression profiling to identify genes that are differentially expressed in the remodeling areas of the R-UUO kidneys compared to repaired regions of the same kidneys, in comparison to UUO and control animals. A better understanding of the key events involved in endogenous renal repair and remodeling may open the way to new interventions aimed at acceleration of renal regeneration and prevention of scarring.

FC-20046

LONG-TERM OVEREXPRESSION OF INTERLEUKIN-10 SUPPRESSES MONOCYTE CHEMOATTRACTANT PROTEIN-1 EXPRESSION AND INFLAMMATION IN A MODEL OF CHRONIC RENAL DISEASE

WEI MU[1], XIAOSEN OUYANG[2], DAVID A LONG[2], CARLOS A RONCAL J[2], ANUPAM AGARWAL[3], OLENA Y GLAUSHAKOVA[2], VINCE A. CHIODO[2], WILLIAM W HAUSWIRTH[2], BERNARDO RODRIGUEZ-ITURBE[4], RICHARD J JOHNSON[1,2]
University of Florida[1], Division of Nephrology, University of Florida, Gainesville, USA[2]; Division of Nephrology, University of Alabama, Birmingham, USA[3]; Division of Nephrology, University of Maracaibo, Maracaibo, Venezuela[4]

Interleukin-10 (IL-10) is a cytokine that plays a pivotal role in the regulation of T-cell immune responses. Previous studies have shown that IL-10 may diminish inflammatory and proliferative responses in animal models of glomerulonephritis. However, to date there have been no studies on the potential benefits of IL-10 in chronic renal disease. Therefore, long-term expression of IL-10 in rats was induced by administration of an adeno-associated virus IL-10 (AAV-IL-10) vector. Control rats received a similar dose of AAV-GFP. 4 weeks after injection, IL-10 levels (measured by ELISA) were significantly elevated (346 ± 64 versus 64 ± 6 pg/ml, IL-10 vs controls, $n = 6$ per group, $p = 0.001$). At this time rats underwent 5/6 nephrectomy, 8 weeks after of surgery, the rate were sacrificed for RNA, protein and immunohistochemical analysis. At sacrifice, IL-10 levels remained 6-fold higher than controls ($p < 0.01$). IL-10 treated animals had improved renal function compared to the control-GFP group with significantly less proteinuria ($p < 0.05$), serum creatinine ($p < 0.05$) and an improved creatinine clearance rate ($p < 0.01$). Renal interstitial infiltration was significantly inhibited by IL-10 administration as assessed by numbers of CD4+, CD8+, ED-1 and OX-62 positive cells (approximately 40–60% reduction, $p < 0.05$). IL-10 administration also decreased renal IFN-γ and IL-2 mRNA (by quantitative real-time PCR) indicating that IL-10 is primarily involved in suppressing the Th1 response in chronic renal disease. Finally, to assess the potential mechanism of reduced inflammation in the IL-10 treated animals, we measured levels of a chemokine, monocyte chemoattractant protein-1 (MCP-1), a potent chemoattractant for macrophages and T-cells. Kidney MCP-1 mRNA and protein levels were suppressed by IL-10 treatment as measured quantitatively by real-time PCR and ELISA respectively ($p < 0.05$). Collectively, these results indicate that IL-10 inhibits the renal interstitial inflammation in chronic renal disease potentially through lowering MCP-1 expression in kidney leading to an improved renal function.

FC-20047

TRANSGLUTANIMANSE (TG) INHIBITION AMELIORATES THE DEVELOPMENT OF EXPERIMENTAL DIABETIC NEPHROPATHY (DN)

LINGHONG HUANG[1], JOHN HAYLOR[1], MARIE FISHER[1], ZOE HAU[1], MARTIN GRIFFIN[2], MEGUID EL NAHAS[1], TIM JOHNSON[1], MARTIN GRIFFIN[2]
Sheffield Kidney Institute[1], Department of Life Sciences, Nottingham Trent University[2]

In kidney disease increased extracellular Tg leads to ε(γ-glutamyl)-lysine crosslinking within the Extracellular Matrix (ECM), altering ECM homeostasis and causing expansion of the basement membranes & scarring. In the acute 5/6th subtotal nephrectomy model of renal scarring, Tg inhibitors maintained renal function by prevention of tissue scarring. Here we demonstrate long term anti-Tg therapy in a chronic rat model of DN.
Following uninephrectomy (UNx) to accelerate DN, 50 mmol/L Tg inhibitor NTU281 was infused continually into the left kidney from an osmotic minipump. Diabetes was induced 7 days later using 35 mg/kg of Streptozotocin (STZ). 4 experiment groups of normal, UNx, untreated diabetics (DM) and DM treated with NTU281 were used. Blood glucose was maintained between 20–25 mmol/L with insulin implants. Blood, urine and tissue samples were collected 1, 4 and 8-months post STZ. Kidney function was assessed by creatinine clearance and renal scarring by image analysis of Masson's Trichrome stained sections. Active TGFβ was measured using a PAI-1 reporter bioassay and specific ECM proteins by immunofluorescence.
Creatinine clearance decreased by 48.6% in DM rats by 8-months. In the NTU281 treated group, it remained similar to the non-diabetic UNx group. An increase in proteinuria was detected in DM rats; however, proteinuria was reduced by >50% in the treated groups at 4 and 8-months. Histological examination of kidneys 8-months post STZ indicated extensive glomerulosclerosis (1.16 ± 0.58) and tubulointerstitial (1.3 ± 0.86) scarring in untreated diabetics. In comparison diabetic animals treated for 8 months with NTU281 had reduced scarring in both the glomeruli (0.25 ± 0.10) and tubulointerstitium (0.29 ± 0.13) resulting from lower deposits of Collagens I, III and IV. This was associated with lower active TGFβ levels.
Tg inhibition from the onset of diabetes in experimental DN improves kidney function and reduces renal scarring over an 8-month period. This type of therapy offers potential for translation to the clinical environment.

FC-20048

EX VIVO PHENOTYPIC SWITCH DETERMINES DAMAGING VERSUS PROTECTIVE EFFECT OF MACROPHAGE IN MURINE ADRIAMYCIN NEPHROSIS

YING WANG[1], YIPING WANG[1], GUOPING ZHENG[2], YUET-CHING TAY, XIAOHONG QIN, DEEPIKA MAHAJAN, STEPHEN I ALEXANDER, DAVID C.H. HARRIS
Centre for Transplantation and Renal Research, The University of Sydney at Westmead Millenium Institute[1]; Centre for Kidney Research Children's Hospital at Westmead NSW 2145 Australia.[2]

Background: Macrophage infiltration is a constant feature of glomerular and tubulointerstitium in both human and experimental kidney disease. We previously showed that depletion of macrophages partially reduced renal structural and functional injury in murine adriamycin nephropathy (AN). Here we investigated whether macrophage phenotype was important in determining its effect on kidney disease in the absence of T and B cells.
Methods: AN was produced in male SCID mice (deficient in T and B cells) by adriamycin (ADR 5.2 mg/kg single/*iv*). Macrophages separated from normal female Balb/C mice were incubated with or without LPS (2.5 µg/ml for 2 hours, Type 1 MΦ) or with IL-4 and IL-13 (10 ng/ml each for 48 hours, Type 2 mΦ). One million cells were transferred into treated mice by a single *iv* at day 5 after ADR. Seven mice in each of groups A (ADR + saline treated), B (ADR + unstimulated MΦ), C (ADR + Type 1 MΦ) and D (ADR + Type 2 MΦ) were sacrificed and renal function and histopathological features were assessed on day 28.
Results: Type 1 MΦ worsened, Type 2 MΦ ameliorated and unstimulated MΦ did not affect renal function and histology compared with the mice on adriamycin alone.

	ADR	Unstimulated MΦ	Type 1 MΦ	Type 2 MΦ
Creatinine Clearance (µL/min)	22.5 ± 6.3	22.6 ± 7.2	12.3 ± 5.3	48.3 ± 6.8
Glomerular Sclerosis (0–3)	1.7 ± 0.5	1.7 ± 0.4	2.6 ± 0.5	0.7 ± 0.3
Interstitial Expansion (0–3)	1.5 ± 0.6	1.6 ± 0.4	2.6 ± 0.7	0.8 ± 0.4
Tubular Atrophy (0–3)	1.5 ± 0.6	1.6 ± 0.4	2.6 ± 0.4	1.0 ± 0.3
Overall Injury (0–3)	1.6 ± 0.7	1.7 ± 0.4	2.5 ± 0.5	1.2 ± 0.6

For all comparisons among unstimuated, Type 1 MΦ and Type 2 MΦ groups (ANOVA), $P < 0.01$.
Conclusions: Reconstitution of Type 1 MΦ but not unstimulated macrophages can substantially augment renal injury in AN, whereas, reconstitution with Type 2 MΦ reduces injury in AN. These results demonstrate that *ex vivo* modulation of macrophage phenotype can directly affect the capacity of these cells to modify renal injury.

Epidemiology and Pathogenesis of Chronic Kidney Disease

FC-20049

PREDOMINANT EFFECTS OF KIDNEY DISEASE (KD) ON EXCESS MORTALITY IN PIMA INDIANS WITH OR WITHOUT TYPE 2 DIABETES

MEDA E. PAVKOV[1], PETER H. BENNETT[1], MAURICE L. SIEVERS[1], JONATHAN KRAKOFF[1], DESMOND E. WILLIAMS[2], WILLIAM C. KNOWLER[1], ROBERT G. NELSON[1],
NIDDK[1], CDC[2]

We examined the effects of KD on mortality from natural causes in non-diabetic and diabetic Pima Indians aged ≥45 years, between 1965–2001.
Deaths per person-years (pyrs) of follow-up were stratified in a time-dependent manner into categories of 1) no proteinuria and normal serum creatinine (SCr), 2) proteinuria (≥0.5 g/g) and normal SCr, 3) high SCr (SCr ≥ 133 µmol/L (1.5 mg/dl) in men, ≥124 µmol/L (1.4 mg/dl) in women), or 4) renal replacement therapy (RRT).

Among 1,990 subjects 55.8% had type 2 diabetes at baseline. In nondiabetic subjects age-sex-adjusted death rates increased from 23.8/1,000 pyrs (95% CI 20.8–26.8) in those without KD to 84.5/1,000 pyrs (95% CI 1.6–167.4) in subjects with high SCr ($p < 0.0001$), with excess mortality primarily from infections. Among diabetic subjects, death rates rose from 25.3/1,000 pyrs (95% CI 22.3–28.2) in subjects without KD, to 209/1,000 pyrs (95% CI 171.1–246.3) in those with RRT ($p < 0.0001$). Death rates were associated with KD regardless of diabetes duration (Figure 1). High SCr was associated with higher death rates from cardiovascular disease (CVD), diabetic nephropathy (DN), and infections.

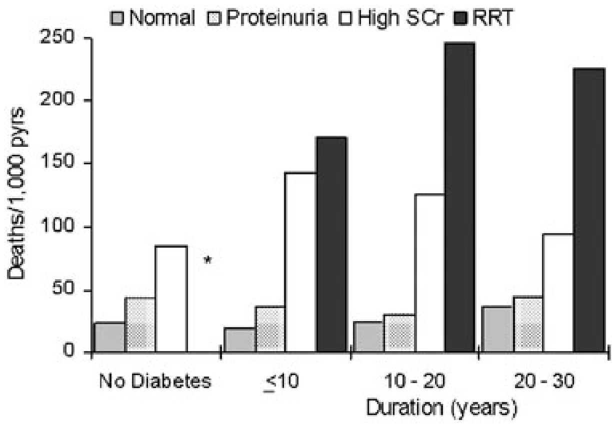


Fig. 1 Death rates from natural causes, stratified by diabetes/duration in four categories of kidney function. There was a strong effect of kidney function in all categories of diabetes duration, but lesser effects of duration for any category of kidney function.* = insufficient data

Conclusion: Death rates increased with worsening kidney function in both nondiabetic and diabetic subjects and were similar in nondiabetic and diabetic subjects without KD. KD was associated with excess mortality from DN, CVD, and infections in diabetic, and from infections in nondiabetic subjects.

FC-20050

THE PROGNOSIS OF CHRONIC KIDNEY DISEASE K/DOQI STAGE 3: A TEN YEAR POPULATION-BASED STUDY OF THE EFFECTS OF GENDER AND AGE

BJØRN ODVAR ERIKSEN[1], OLE CHR. INGEBRETSEN[2]
Department of Nephrology, University Hospital of North Norway[1]; Department of Clinical Chemistry, University Hospital of North Norway, Tromsø, Norway[2]

The increasing demand for renal replacement therapy makes it important to investigate the prognoses of the earlier stages of chronic kidney disease (CKD). We examined change in glomerular filtration rate (GFR), and patient and renal survival in CKD K/DOQI stage 3 in Tromsø, a well-defined European community with a population of 58,000.

All patients with estimated GFR between 30 and 59 ml/min/1.73 m^2 for more than three months during a ten year study period were identified from a complete database of all 248,560 measurements of serum creatinine made in the community in the study period. Change in GFR was estimated for each patient using a linear model. A complete follow-up of patient and renal survival was obtained from hospital databases.

3047 patients were included. The mean number of measurements of creatinine for each patient was 14; the mean observation time 50 months. Mean change in GFR was −1.02 ml/min/1.73 m^2/year (SD 8.22). 61% experienced a decline in GFR. Ten year cumulative incidence of renal failure was 0.04 (95% CI 0.03 to 0.06); mortality 0.51 (95% CI 0.48 to 0.55). Slower decline in GFR and better patient and renal survival were seen in women. Increasing age was associated with faster decline, higher mortality and a lower cumulative incidence of renal failure because it was pre-empted by death.

Our population-based estimate of GFR decline was markedly slower than in previously studied selected CKD patients. Different prognoses in identifiable subgroups make it difficult to establish guidelines with a uniform approach to all patients. Risk factors for predicting which patients have progressive disease should be included in the definitions of stages, as i.e. proteinuria. Although included in the definition of K/DOQI stages 1 and 2, definitions of the advanced stages have been made without taking proteinuria into account. This needs to be reconsidered.

FC-20051

RACE DIFFERENCES IN THE DISTRIBUTION OF NOVEL CARDIOVASCULAR RISK FACTORS AMONG PERSONS WITH CHRONIC KIDNEY DISEASE: FINDINGS FROM THE THIRD NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY

HOANG THANH NGUYEN[1], MURTHY V.R. BHAMIDIPATI[1], AUSTIN G. STACK[2]
Department of Internal Medicine, Division of Renal Diseases and Hypertension, University of Texas at Houston Medical School, U.S.A.[1]; Regional Kidney Center, Letterkenny and Sligo General Hospitals, North Western Health Board, Ireland[2]

The prevalence of clinical coronary disease differs by race among persons with chronic kidney disease (CKD). Whether these can be accounted for by differences in the distribution of novel cardiovascular risk factors is unclear.

We compared the prevalence of novel inflammatory [C-reactive protein (CRP), white blood cell count (WBC)] and prothrombotic [apolipoprotein A1 (Apo A1), and apolipoprotein B (Apo B), homocysteine (HCY), lipoprotein (a) (Lp (a)), and plasma fibrinogen] risk factors among Whites, Blacks and Hispanics with reduced kidney function in the Third National Health and Nutrition Examination Survey, a population-based survey of the non-institutionalized U.S. population. CKD was defined as a glomerular filtration rate <60 ml/min per 1.73 m^2 from the 4-variable Modification of Diet in Renal Disease Study equation. Pathological levels for each factor were defined as those within the highest quartile with the exception of ApoA1 (lowest quartile) and CRP (≥10.0 mg/L). Age-standardized prevalence for each factor was computed and compared by race while weighted logistic regression was used to compare the odds-ratios.

Age-Standardized Prevalence of Pathological levels of Novel Cardiovascular Risk-Factors by GFR and Race

Risk-Factors	N	>60 ml/min			CKD (<60 ml/min)			p-value*
		White	Black	Hispanic	White	Black	Hispanic	
CRP (>10 mg/L)	15,200	6.8	12.6	8.4	17.3	18.8	10.4	NS
WBC (>75th)	15,033	25.4	18.1	29.4	41.9	17.3	49.5	<.005
Fibrinogen (>75th)	8,844	22.6	31.2	24.7	39.0	61.1	73.2	<.005
Apo A1 (<25th)	7,343	24.4	16.8	27.3	45.5	27.1	24.2	NS
Apo B (>75th)	7,360	36.6	22.5	29.4	61.2	31.8	44.7	<.05
Lp(a) (>75th)	7,769	20.9	57.8	15.6	22.6	60.6	40.8	NS
HCY (>75th)	6,790	24.8	25.5	19.7	77.9	49.2	95.3	<.0001

*p-value is from t-text of polynominal linear or quadratic contrast across race categories for the CKD population NS = Not statistically significant.

Subjects with CKD had higher prevalence of abnormal levels of almost all risk factors than subjects with GFR >60. Pathological elevations in WBC, fibrinogen and HCY were greatest in Hispanics compared with other races. Conversely, pathological levels of CRP and ApoA1 were lowest in Hispanics compared with other races. Blacks had the lowest prevalence of pathological levels of Apo B. Compared to Whites, Blacks were 1.8 and 3.7 times more likely to have abnormal levels of fibrinogen and Lp (a) but 38% less likely to have abnormal level of WBC, while Hispanics were 1.7 and 2.6 times more likely to have abnormal levels of Apo A1 and fibrinogen in the CKD population. Whether these translate into measurable differences in cardiovascular event rates needs further study.

FC-20052

ASYMMETRIC DIMETHYLARGININE (ADMA) AND PROGRESSION OF CHRONIC KIDNEY DISEASE: THE MILD TO MODERATE KIDNEY FAILURE (MMKF) STUDY

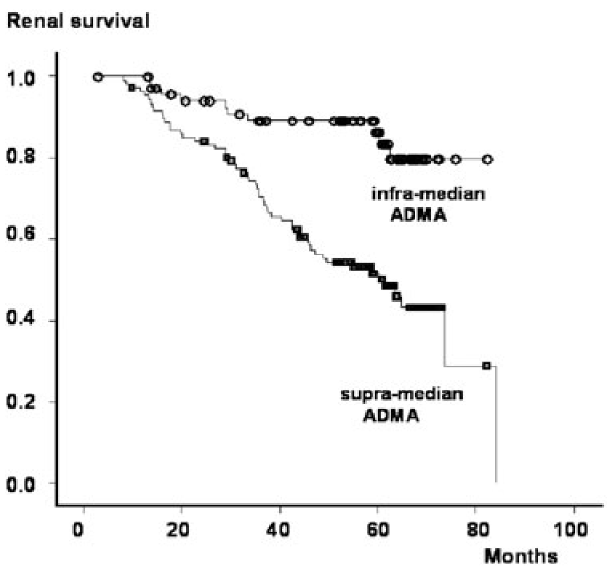
JAN T. KIELSTEIN[1], FLORIAN KRONENBERG[2], DANILO FLISER[1], STEFANIE M BODE-BOEGER[3], EBERHARDT RITZ[4]

Department of Nephrology, Medical School Hannover, Germany[1]; Institute Med. Biol. & Hum. Gen., University of Innsbruck, Innsbruck, Austria[2]; Institute of Clinical Pharmacology, University Hospital Ott-von-Guericke University, Magdeburg, Germany[3]; Department of Internal Medicine, Ruperto-Carola University, Heidelberg, Germnay[4]

Background: Increased blood levels of the endogenous NOS inhibitor asymmetric dimethylarginine (ADMA) have been proposed to contribute to the progression of chronic kidney disease (CKD). We assessed the influence of ADMA and other putative progression factors in a prospective multi-centre study in 227 Caucasoid patients with non-diabetic kidney disease.

Study population and Methods: Patients were recruited from eight nephrology departments in Germany, Austria, and South Tirol. Major exclusion criteria were diabetes mellitus, immunosuppressive treatment, serum creatinine above 6 mg/dl, nephrotic syndrome and malignancy. ADMA concentrations were measured with liquid chromatography-mass spectrometry method. In addition, glomerular filtration rate (GFR) was assessed using the iod-thalamate clearance technique. After baseline examination patients were followed prospectively until doubling of serum creatinine, terminal renal failure necessitating renal replacement therapy, or end of the 82 months observation period.

Results: At baseline plasma ADMA levels were significantly correlated with age ($r = 0.281$), serum creatinine ($r = 0.595$), GFR ($r = -0.591$), proteinuria ($r = 0.184$), hemoglobin ($r = -0.336$) and PTH ($r = 0.586$) (all $p < 0.01$). A total of 177 patients (78%) in the baseline cohort could be assessed during the follow-up period. The median follow-up time was 54 [1–82] months, and during this period 65 patients reached a progression endpoint. We performed Cox regression with variables which were significantly different in patients who progressed during follow-up only baseline serum creatinine (OR = 30.36; 95% CI 13.09–70.34; $p < 0.001$) and ADMA (OR = 6.35; 95% CI 2.94–13.70; $p < 0.006$) were significantly associated with progression. In patients with supra-median ADMA values progression was significantly ($p < 0.0001$) faster, and their mean follow-up time to a progression endpoint was to 54.6 (95% CI 48.9–60.4) months as compared to 71.3 (95% CI 65.7–76.9) months in patients with infra-median ADMA levels (Figure).



Conclusion: The results of this prospective study in a sizable cohort of patients with non-diabetic kidney disease point to ADMA as a novel risk factor in the progression of renal disease.

FC-20053

IMPACT OF DIABETIC NEPHROPATHY AND ANGIOTENSIN II RECEPTOR BLOCKADE ON URINARY POLY-PEPTIDE PATTERNS

KASPER ROSSING[1], HARALD MISCHAK[2], HANS-HENRIK PARVING[3], PER K. CHRISTENSEN[1], MICHAEL WALDEN[4], MEIKE HILLMANN[4], THORSTEN KAISER[4]

Steno Diabetes Center[1], Mosaiques diagnostics & therapeutics AG, Hannover, Germany[2], Steno Diabetes Center, Niels Steensen Vej 2, Gentofte, Denmark[3], Mosaiques diagnostics & therapeutics[4]

Background: New insights into the pathogenesis and treatment of diabetic renal disease may emerge from recent advances in proteomics using high-throughput mass spectrometry (MS) of urine.

Methods: Using a combination of online capillary electrophoresis(CE) and MS we evaluated urinary polypeptide patterns(UPP) in 4 groups of type 2 diabetic patients matched for age, gender and diabetes duration including; 20 normoalbuminuric patients with and 20 without diabetic retinopathy(DR), 20 microalbuminuric patients with DR and 18 macroalbuminuric patients with DR. Furthermore, changes in UPP during treatment with the angiotensin II receptor blocker candesartan were evaluated in the macroalbuminuric patients in a randomized double-blind, cross-over trial where each patient received treatment with placebo, candesartan 8, 16 and 32 mg daily each for two months.

Results: Overall 4551 different polypeptides were found in the samples. UPPs were comparable in normo- (with and without DR) and microalbuminuric patients whereas distinct differences were found in macroalbuminuric patients. Differences in UPP between normo- and macroalbuminuric patients permitted the establishment of a 'diabetic renal damage' (DRD) pattern consisting of 113 polypeptides. Eleven of these polypeptides had been sequenced and identified. Candesartan treatment in macroalbuminuric patients significantly changed 15 of the 113 polypeptides in the DRD pattern towards levels in normoalbuminuric patients. Change in the DRD pattern was not candesartan dose-dependent but individual changes correlated with changes in urinary albumin excretion at each dose level.

Conclusions: CE-MS serves as a fast and sensitive tool for identification of biomarkers and urinary polypeptide patterns specific for macroalbuminuric type 2 diabetic patients and may be used to explore and monitor renoprotective effects of angiotensin II receptor blockade.

FC-20054

UP-REGULATION IN THE KIDNEY AND GENETIC POLYMORPHISM OF MUC20, A REGULATOR OF MET SIGNALING CASCADE, IN PATIENTS WITH IGA NEPHROPATHY

ICHIEI NARITA[1], BASSAM ALCHI[2], FUMINORI SATO[3], DAISUKE KONDO, ASA OGAWA, YUTAKA TSUBATA, MINORU SAKATSUME, TADASHI YAMAMOTO, FUMITAKE GEJYO

Division Of Clinical Nephrology And Rheumatology, Niigata University, Japan[1]; Division of Clinical Nephrology and Rheumatology and Institute of Nephrology[2], Niigata University Graduate School of Medical and Dental Sciences[3]

MUC20, mucin protein 20, was isolated as a gene up-regulated in the renal tissue of IgA nephropathy (IgAN). The functional analyses of MUC20 have demonstrated that it is a novel negative regulator of the HGF-induced Grb2-Ras pathway. It has been suggested that oligomerization of MUC20 caused by either an overproduction or an unknown factor(s) leads to the association with Met. In human MUC20, the repeat numbers of the extracellular tandem domain, which may have an influence on the oligomerization, showed a divergence, with 2 to 6-repeat types in several human cell lines. To clarify the role of MUC20 in human kidney diseases, we analyzed the expression of MUC20 in the kidney tissues of primary glomerulonephritis by in situ hybridization and investigated a possible association of the tandem repeat polymorphism of MUC20 with renal survival in 236 patients with histologically- proven IgAN. In normal kidneys, MUC20 expression was localized only in distal tubules. In addition, glomerular podocytes and Bowman's capsule were positive for MUC20. In contrast, it was expressed in proximal tubules in addition to distal tubules in renal tissues of IgAN, whereas change in glomerular expression was not significant. The prognosis of IgAN patient with 5 or 6 of tandem repeat of MUC20 was significantly better than those without ($P = 0.001$). The tandem repeat polymorphism of MUC20 was an independent risk factor for progression even after adjusting with other clinical risk factors, including hypertension, urinary protein excretion. MUC20 regulates the Met signaling cascade, which is implicated in tubular repair and regeneration under pathological condition in glomerulonephritis. Factors that regulate the function of MUC20 may be useful therapeutic agent for progression of renal injury.

Nephron Function

FC-10055

COLLECTING-DUCT-SPECIFIC DELETION OF PPARγ BLOCKS THIAZOLIDINEDIONE-INDUCED FLUID RETENTION

TIANXIN YANG[1], AIHUA ZHANG[1], RAOUL D. NELSON[1], FRANK J. GONZALEZ[2], DONALD E. KOHAN[1], HUI ZHANG[1]
University of Utah[1], National Institute of Health[2]

The peroxisome proliferator-activated receptor subtype g (PPARg) ligands, namely the synthetic insulin-sensitizing thiazolidinedione compounds (TZDs), have demonstrated great potential in the treatment of type II diabetes. However, their clinical applicability is limited by a common and serious side effect of edema. To address the potential role of the distal nephron in TZD-induced fluid retention, the present study generated mice with collecting duct (CD)-specific deletion of PPARg gene (termed PPARg KO) by genetic cross between PPARg floxed ($PPAR\gamma^{f/f}$) and AQP2-CreTag mice. A 7-day rosiglitazone (RGZ) treatment consistently increased body weight in $PPAR\gamma^{f/f}$ mice but not in PPARγ KO mice. Following the RGZ treatment, $PPAR\gamma^{f/f}$ mice exhibited severe plasma volume expansion as reflected by significant decreases in hematocrit and plasma aldosterone levels, and significant increases in plasma volume measured by Evans blue technique. In contrast, the RGZ-induced plasma volume expansion was remarkably blunted in PPARγ KO mice. The volume expansion in RGZ-treated $PPAR\gamma^{f/f}$ mice was preceded by a positive sodium balance, whereas PPARγ KO mice maintained normal sodium balance throughout the entire experimental period. RGZ significantly stimulated sodium transport as reflected by decreases in the transepithelial resistance in the primary culture of CD cell-derived $PPAR\gamma^{f/f}$ mice, while the stimulation was completely blocked in PPARγ KO cells. We conclude that TZD-induced fluid retention is mediated by activation of PPARγ in the CD.

FC-10056

REGULATION OF RENIN SECRETION IN MICE WITH CRE-RECOMBINASE-MEDIATED DELETION OF JUXTAGLOMERULAR GSALPHA

SOO MI KIM[1], LIMENG CHEN[1], YUNING HUANG, MIN CHEN[1], MARIA LUISA SEQUEIRA LOPEZ[2], R. ARIEL GOMEZ[2], S. LEE WEINSTEIN[1], JOSIE P. BRIGGS[1], JURGEN SCHNERMANN[1]
NIDDK, NIH[1], University of Virginia[2]

The present experiments were performed to assess the role of Gsa as a mandatory transducer of G protein-coupled receptor activation in the regulation of renin secretion. Since a complete Gsa knockout is embryonic lethal, we generated mice in which Gsa was eliminated selectively in renin-expressing cells by cell-specific cre-mediated recombination. These mice were the offspring of a cross between mice heterozygous for a knockin of cre recombinase into the Ren1d locus placing cre under the control of the renin promoter (Sequeira Lopez et al., Dev. Cell 6:719, 2004) and mice in which exon 1 of the Gsa gene was flanked by LoxP sites (Chen a.Weinstein, NIDDK, unpublished). Ren1d-Cre/GsF.F mice were viable and showed no obvious anatomical abnormalities. Cre-mediated DNA recombination and inactivation of Gsa was found in the kidney cortex as well as in the renal medulla. Basal plasma renin concentration (PRC; ng AngI/ml/hr) was extremely low, and it did not significantly change with the administration of furosemide (43 ± 28 vs. 81 ± 94), isoproterenol (76 ± 41 vs. 387 ± 129), or hydralazine (172 ± 54 vs. 260 ± 296). In control Ren1d/GsF.F mice furosemide increased PRC from 926 ± 211 to 5652 ± 1888, isoproterenol from 1041 ± 257 to 1556 ± 384, and hydralazine from 1007 ± 258 to 1672 ± 416 ($p > .05$ for all interventions). In agreement with medullary Gsa inactivation Ren1d-cre/GsF.F mice had an AVP-resistant concentrating defect. We conclude that ambient as well as macula densa- and baroreceptor-dependent renin release is dominated by Gs-coupled receptor signaling. Thus, cyclic AMP appears to be the major intracellular messenger regulating renin synthesis and release.

FC-10057

MACULA DENSA REGULATION OF RENIN SECRETION AND PREGLOMERULAR RESISTANCE IN MICE LACKING THE B-ISOFORM OF THE NA/K/2CL COTRANSPORTER NKCC2

HAYO CASTROP[1], MONA OPPERMANN[1], YUNING HUANG[1], DIANE MIZEL[1], CHUXIA DENG[1], JOSIE P. BRIGGS[1], JURGEN SCHNERMANN[1]
NIDDK, NIH[1]

NaCl transport by NKCC2 in the macula densa (MD) is an early step in the mechanism by which changes in luminal NaCl concentration are translated into changes of renin secretion and preglomerular resistance. To further address these questions we generated mice lacking the B-isoform of NKCC2 believed to be mainly expressed in the MD. Genomic DNA analysis showed predicted mutations of NKCC2B-specific sequences in NKCC2B-/- mice. Sequencing of transcripts revealed correctly spliced NKCC2A and NKCC2F mRNA at normal expression sites (NKCC2A: OM outer stripe = OM inner stripe > cortex; NKCC2F: OM inner stripe >> OM outer stripe >>>> cortex). Expression of NKCC2F was reduced in NKCC2B-/- mice. Two mutated NKCC2B transcripts were found in NKCC2B-/- mice predicted to result in a truncated and non-functional NKCC2B protein. NKCC2B-/- mice were viable and showed no morphological abnormalities. Ambient urine osmolarity was lower in NKCC2B-/- than in wild type controls (1243 ± 128 vs.1844 ± 149 mosm/l; n = 21 and 15; p = .03 =). Basal plasma renin concentration (PRC; ng angiotensin I/ml/hr) was reduced in NKCC2B-/- (1040 ± 135 vs. 1488 ± 136; n = 12 and 16; p = .03), while increases of PRC with a low salt diet (0.003% NaCl) and decreases of PRC with a high salt diet (8% NaCl) were maintained. In loops of Henle perfused in vivo with 6 nl/min, distal Cl concentration was higher (70 ± 4 vs. 43 ± 3.5 mEq/l; $p < .0001$) and Cl absorption was lower in NKCC2B-/- than wild type mice (653 ± 17 vs. 747 ± 12 pEq/min; p = .00015). Maximum tubuloglomerular feedback responses were not different between NKCC2B-/- and wild type mice, but TGF curves were right-shifted. We conclude that absence of NKCC2B reduces the diluting capacity of the TAL, reduces net Cl absorption along the loop of Henle, and desensitizes the TGF response.

FC-10058

N-TERMINAL SORTING SIGNAL MEDIATES POLARIZED TRAFFICKING OF AQUAPORIN 3 (AQP3) WATER CHANNEL

TATEMITSU RAI[1], SEI SASAKI[1], SHINICHI UCHIDA[1]
Tokyo Medical and Dental University[1]

Epithelial renal collecting duct cells express multiple types of aquaporin (AQP) water channels in a polarized fashion. AQP2 is specifically targeted to the apical cell domain, whereas AQP3 and AQP4 are expressed on the basolateral membrane. Although it is crucial that different types of AQPs are sorted to their proper polarized membrane domains for efficient cell function, little is known about the molecular mechanisms that govern the membrane targeting of AQPs. In the present study, we examined the polarized trafficking and surface expression of AQP3 in MDCK cells to identify regions that specify polarized targeting. Human AQP3 cDNA was subcloned into pcDNA3.1 expression vector.
(Invitrogen) and mutations were introduced by Quikchange Mutagenesis kit (Stratagene). When expressed in MDCK cells, wild type AQP3 was targeted to the basolateral membrane resembling its proper localization in vivo. A potential sorting signal consisting of tyrosine and dileucine based motifs was identified in the N-terminal of AQP3. When mutations were introduced in the sorting signal, basolateral targeting of mutant AQP3s was interfered and mutant AQP3s remained in the cytoplasm. Next, AQP2-AQP3 chimeras were generated by Seamless cloning kit (Stratagene) in which the entire N-terminal of AQP2 was substitute to that of AQP3. AQP2 with the N-terminal of AQP3 was consitutively mis-localized in the basolateral membrane, whereas mutations in the N-terminal signal abolished the effect. From these results, we conclude that the N-terminal sorting signal mediates basolateral targeting of AQP3.

FC-10059

EFFECTS OF VANADATE AND LOW POTASSIUM DIET ON RENAL NA,K-ATPASE AND H,K-ATPASE PROTEIN EXPRESSION IN RAT

RATANAPORN JERAWATANA[1], WIPAWEE KITTIKOWIT[2], SOMCHAI EIAM-ONG[3], SOMCHIT EIAM-ONG[2]
Inter-department of Physiology, Graduate School, Chulalongkorn University, Bangkok 10330, Thailand[1], Department of Pathology, Faculty of Medicine, Chulalongkorn University[2], Department of Physiology, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Thailand[3]

Both vanadate and potassium depletion could alter renal Na,K-ATPase and H,K-ATPase activity. This study was conducted to investigate the effects of vanadate and low potassium diet on Na,K-ATPase and H,K-ATPase protein expression. Male Wistar rats were intraperitoneally injected with normal saline solution (NSS) or vanadate (V; 5 mg/kg. BW). Each group was received either normal-potassium (NK) or low-potassium (LK) diet. The treatments were performed for 10 days. On experimental due date, 24-hr urine and blood samples were collected for measurement of vanadium, electrolytes, blood urea nitrogen, creatinine, and creatinine clearance. The kidneys were removed and determined for vanadium concentration, as well as were fixed for measurement of Na,K-ATPase and H,K-ATPase (alpha1 and alpha2 isoforms) protein expression. Vanadate administration significantly increased vanadium levels in all parts studied in both NK and LK groups. By immunohistochemistry, the main staining of Na,K-ATPase protein expression was in distal tubule and collecting duct. The expression was increased by LK but reduced by V. LK in V treated rats could not restore the expression. For H,K-ATPase, in cortex, LK could enhance the expression of both alpha1 and alpha2 at luminal membrane of collecting duct. In medulla, LK slightly increased alpha1, whereas no expression of alpha2 protein was noted. Vanadate had no effect on both isoforms of H,K-ATPase expression. Treatment of V + LK caused a progressive hypokalemia, azotemia and natriuresis. LK with NSS or V significantly reduced blood pCO2 while blood pH was remained normal. Fractional excretion of potassium, chloride, and bicarbonate were increased in V + LK animal. Urine flow rate were comparable in all groups. These findings are the first evidence showing that LK could not restore the suppressive effect of V on renal Na,K-ATPase protein expression. Vanadate has no influence on renal H,K-ATPase protein expression. LK still plays an important stimulus on increasing of H,K-ATPase protein expression.

FC-10060

COLLECTRIN IS A NOVEL TARGET OF HNF-1 IN RENAL COLLECTING DUCT CELLS

YANLING ZHANG[1], JUN WADA[1], AKIHIRO YASUHARA[1], JUN EGUCHI[1], IZUMI HASHIMOTO[1], KAZUYA YAMAGATA[2], KENICHI SHIKATA[1], HIROFUMI MAKINO[1]
Okayama University Graduate School of Medicine and Dentistry[1], Osaka University[2]

We identified the gene that encodes collectrin, a homologue of angiotensin-converting enzyme 2 (ACE2), which is upregulated in 5/6 ablated mouse kidneys at hypertrophic phase (Zhang H et al. *Kidney Int*, 1999; Zhang H et al. *J Biol Chem*, 2001). Collectrin, a novel member of ACE gene family, is membrane bound protein and exclusively localized and expressed in the collecting duct cells and mouse inner medulla collecting duct cells (mIMCD3). Collectrin lacks a catalytic domain, which indicates that there might be unknown functions besides simple peptide hydrolysis like ACE. Analysis of transcription factor binding sites in the promotor regions of human, rat, and mouse collectrin reveals the presence of hepatocyte nuclear factor-1 consensus binding sequences. We confirmed the specific binding of HNF-1a and HNF-1b to the putative HNF-1 binding site of collectrin gene by electrophoretic mobility shift assay (EMSA) in mIMCD3 cells. We subcloned a 322-bp of the 5′ flanking promoter region of the human collectrin gene into pGL3 Luciferase Reporter vector (Collectrin-pGL3) and co-transfected it with HNF-1a-pcDNA3.1(+) and/or HNF-1b-pcDNA3.1(+) into mIMCD3 cells and luciferase activity assays were performed using the Dual Luciferase Reporter Assay System. HNF-1a, HNF-1b, and co-transfection of HNF-1a and HNF-1b stimulated the promoter activity by 11.1 ± 1.9-, 2.4 ± 0.5- and 13.2 ± 0.7-fold compared with Collectrin-pGL3, respectively. We established stably expressing mIMCD3 cell lines by transfection with HNF-1a-pcDNA3.1(+) and selection in the presence of geneticin. By analyzing several cell lines, Northern and Western blot analyses reveled that collectrin mRNA and protein was up-regulated in the HNF-1a highly expressing stable cell lines. These results suggest that HNF-1a and HNF-1b play important roles in the transcriptional regulation of collectrin and thus collectrin is a novel target of HNF-1 in collecting duct cells.

POSTER COMMUNICATIONS

THEME 1: FUNDAMENTALS OF INTEGRATIVE BIOLOGY

Transport Physiology

W-PO10001

EVALUATION OF THE HEMODYNAMIC PARAMETERS DURING NORMAL PREGNANCY IN RATS: ROLE OF RENAL WATER AND SALT TRANSPORTERS

MIRIAN A BOIM[1], NAYDA P ABREU[1], WELLINGTON QUINTINO[1], JOISSE C.B. TARDIN[1], RUY R. CAMPOS[1], CASSIA T. BERGAMASCHI[1], NESTOR SCHOR[1]
Universidade Federal de São Paulo[1]

Water and salt retention is a typical condition during normal pregnancy. The kidneys are involved in the extracellular volume (ECV) expansion. Thus the aim of the present study was to evaluate if the alterations in ECV observed during normal pregnancy is paralleled with changes in the mRNA expression levels of renal electrolytes and water transporters. Pregnancy was induced in normal Wistar rats and was detected by the presence of vaginal sperm (group P, n = 6). Age matched virgin rats served as control (C, n = 6). The hemodynamic parameters including blood pressure (BP), heart rate (HR), stroke volume (SV), total peripheral resistence (TPR) and cardiac output (CO) were analyzed at 14th day of pregnancy. The CO was estimated by the termodilution method. The mRNA expression levels of Na/H exchanger (NHE3), Na/K/2Cl cotransporter (BSC) and the aquaporin 2 (AQP2) were analyzed in the kidney tissue by RT-PCR technique. Results were expressed as densitometric units using b actin mRNA as the endogenous control. CO increased in P group compared with C group (107 ± 7 vs 84 ± 1 ml/mim, p = 0.01) without any change in blood pressure, followed by a decrease in TPR (1.4 ± 0.07 1.2 ± 0.1 (mmHg/ml/min). There was an increase in both diuresis and sodium excretion in P group. These alterations were followed by an increase in the mRNA levels for NHE3 (79%), BSC (100%) and AQP2 (250%) compared with C group. Similar to humans, the pregnant rats presented a rise in CO indicating an expansion of ECV with no changes in BP. The upregulation of Na and water transporters in the kidney may be involved in ECV expansion during this especial physiological condition.

W-PO10002

IMMUNOCYTOCHEMICAL LOCALIZATION OF A NOVEL VASODILATOR PEPTIDE, ADRENOMEDULLIN 2/INTERMEDIN, IN HUMAN KIDNEY

KAZUHIRO TAKAHASHI[1], KUMI KIKUCHI[2], TOMOKO URABE[3], KIICHIRO NAKAJIMA[3], HIRONOBU SASANO[4], KAZUHITO TOTSUNE[5], OSAMU MURAKAMI[2]
Department of Molecular Biology & Applied Physiology, Tohoku University School of Medicine, Sendai, Japan[1], Department of Medicine Tohoku University School of Medicine[2], Peptide Institute[3], Department of Pathology[4], Department of Clinical Pharmacology[5]

Adrenomedullin 2/intermedin (AM2/IMD) is a novel member of the calcitonin/calcitonin gene-related peptide (CGRP) peptide family, that was identified by searching the genome database. AM2/IMD has a vasodilator action, and anti-diuretic and antinatriuretic effects in mice. Immunocytochemical localization of AM2/IMD in human tissues has not been studied in detail. We studied expression of AM2/IMD in human kidney as well as hypothalamus and heart by immunocytochemistry. Antiserum against human AM2/IMD was raised in a rabbit by injecting human AM2/IMD (1–7) conjugated with bovine thyroglobulin in the Peptide Institute Inc. (Osaka, Japan). In the human kidney obtained at autopsy, renal tubular cells were positively immunostained with AM2/IMD, whereas neither glomeruli nor vasculature in the kidney were immunostained. AM2/IMD-immunoreactive cell bodies are found in the paraventricular and supraoptic nuclei of human hypothalamus. Both parvocellular and magnocellular cells in the paravetricular nucleus are immunostained with AM2/IMD. Immunostaining of serial sections showed colocalization of AM2/IMD and vasopressin in these cells. Myocardial cells of the heart were positively immunostained. These results indicate that AM2/intermedin is expressed in the renal tubular cells, suggesting that this peptide may regulate water-electrolyte metabolism and renal circulation in the kidney. Furthermore, expression in the hypothalamus and heart suggest that this novel peptide may be related to the central and peripheral regulation of the circulation and water-electrolyte metabolism.

W-PO10003

IMCD WATER CHANNEL AQUAPORIN 2 EXPRESSION WAS INCREASED BY GLUCAGON IN NORMAL RATS

ANTONIO-J MAGALDI[1], Y YANO[1], AC RODRIGUES[1], L C ANDRADE[1]
Clinical Hospital-University of São Paulo-LIM[1]

In animals supplied with a high protein diet the urinary concentrating capacity is enhanced and the release of Glucagon(Gl) is also increased. It is known that Gl participates in water excretion by the kidney after a protein meal and an i.v. amino acids infusion. However, the direct effect of this hormone on perfused (IMCD), is not well defined. Thus, the effect of Gl was examined in water transport in isolated IMCD perfused 'in vitro'from normal rats. Osmotic Water Permeability (Pfmm/s), was studied at 37ºC and pH 7.4 in normal rat IMCD($n = 26$) perfused and bathed with Ringer/HCO_3. Gl (10^{-7} M) added to the bath fluid in absence of Vasopressin (Vp), enhanced the Pf from 4.38?.40 to 11.16?.44 ($p < 0.01$). Adding Gl 10^{-8}, 10^{-7} and 10^{-6} M, the Pf responded in a dose-dependent manner. The Protein kinase A inhibitor H8 blocked the Gl effect. The specific Gl inhibitor, des-His[1]-[Glu[9]] glucagon (10^{-7} M), blocked the Gl stimulated Pf but not the Vasopressin(Vp) stimulated Pf. The cAMP level enhanced from the control 1.24?.39 fm/mg prot to 59.70?5.18 after Gl 10^{-7} M in an IMCD tubule suspension. These results are in agreement with the subsequent immunobloting studies that indicated an increase in AQP2 protein abundance of 27% compared with the controls(cont-100.0?3.9 vs Gl 127.53, $p = 0.0035$) in membrane fraction extracted from IMCD tubule suspension, incubated with 10^{-6} M Gl. Our data showed that: 1- Gl increased water absorption in a dose-dependent manner; 2- The anti-Gl blocked the action of Gl but not the action of Vp; 3- Gl stimulated the cAMP generation; 4- Gl increased the AQP2 water channel protein expression, leading us to conclude that Gl affects water absorption by utilizing a Gl receptor(rather than a Vp receptor) increasing the AQP2 protein expression.

W-PO10004

THE ROLE OF RENAL DOPAMINE SYSTEM IN SODIUM RETENTION OF PAN AND HGCL2-INDUCED NEPHROSIS

BENEDITA SAMPAIO-MAIA[1], M MOREIRA-RODRIGUES[2], O AZEVEDO-SILVA[2], P SERRAO[1], M PESTANA[2]
Inst Pharmacology and Therapeutics[1], Unit of Research and Development of Nephrology, Faculty of Medicine, Porto, Portugal[2]

This study evaluated the role of renal dopamine in the sodium retention of puromycin aminonucleoside (PAN) and $HgCl_2$-induced nephrosis. Sprague-Dawley rats (Harlan, Spain) weighing 150 g received PAN (150 mg/kg bw, ip; $n = 9$) or the vehicle (0.9% NaCl, ip; $n = 9$) on day 0. Brown-Norway rats (Harlan, Spain) weighing 150 g received $HgCl_2$ (1 mg/kg bw, sc; $n = 9$) or the vehicle (0.9% NaCl, sc; $n = 9$) on days 0, 2, 4, 7, 9 and 11. Twenty-four hours urine was collected for determination of sodium and dopamine. The renal aromatic *L*-amino acid decarboxylase activity (AADC), the enzyme responsible for dopamine synthesis, was evaluated in PAN-treated rats on days 7 and 14 and in $HgCl_2$-treated rats on days 7, 14 and 21. During greatest sodium retention and ascites accumulation (day 7 for PAN-treated rats and day 14 for $HgCl_2$-treated animals) the nephrotic and control rats were submitted to a 5% bw volume expansion (VE) with saline and the effects of the D_1 agonist (fenoldopam, 10 μg/kg bw/min) on the natriuresis and on the Na^+,K^+-ATPase activity in the renal proximal tubules was evaluated. A marked decrease in renal AADC activity was observed in PAN and $HgCl_2$-treated rats on days 7, 14 and 21. This was accompanied in PAN and $HgCl_2$-treated rats with a marked decrease in the daily urinary excretion of dopamine. The VE was accompanied with a blunted natriuretic response in both PAN and $HgCl_2$-treated rats. During fenoldopam infusion the natriuretic response to VE was significantly increased in control rats but was not altered in both PAN and $HgCl_2$-treated animals. The fenoldopam-induced inhibition of Na^+,K^+-ATPase activity in renal proximal tubules was well preserved in either PAN or $HgCl_2$-treated rats. We conclude that a blunted renal dopaminergic tonus is observed in both PAN and $HgCl_2$-induced nephrosis. This may contribute to increase the proximal sodium retention in a state of proteinuria. (Grant-POCTI/FCB/45660/2002-FCT)

W-PO10005

TUBULAR TRANSPORTERS AND CLEARANCE OF ADEFOVIR

SERVAIS A[1], LECHAT P[1], ZAHR N[1], URIEN S[1], AYMARD G[1], JAUDON MC[1], DERAY G[1], ISNARD BAGNIS C[1]
Pitié-Salpêtrière University Hospital[1]

Introduction: Adefovir was shown to be effective in hepatitis B virus therapy. It is transported *in vitro* by two tubular transporters, the organic anion transporter (OAT1) and the multidrug resistant protein (MRP2). We studied adefovir clearance *in vivo* in rat after pharmacological inhibition of transporters by probenecid and in mutant transport-deficient rats (TR-), in which MRP2 is lacking.
Methods: After treatment by probenecid or placebo, adefovir (10 or 30 mg/kg) was administered via tail artery to normal Wistar rats or to TR- rats. Sequential blood and urine samples were collected during 48 hours and pharmacokinetics was studied via population modeling (NONMEM).
Results: The fraction of drug excreted in the urine was low (table 1). Renal clearance of adefovir in control TR- rats (0.13 +/– 0.07 l/h) was not different from normal control but it was significantly lower ($p < 0.05$) in probenecid TR- rats (0.03 +/– 0.02 l/h) than in normal control (0.09 +/– 0.05 l/h), in normal probenecid (0.10 +/– 0.07 l/h) and in TR- control rats (0.13 +/– 0.07 l/h). Non renal clearance of adefovir 10 mg/kg increased significantly in probenecid normal rats (0.47 +/– 0.10 l/h) compared to normal controls (0.29 +/– 0.11 l/h, $p < 0.0001$).
Conclusion: *In vivo* in rats MRP2 mutation alone did not affect adefovir clearance suggesting that MRP2 does not play a critical role in the secretion of adefovir. Additional pharmacological inhibition of transporters decreased renal clearance which may reflect inhibition of compensating transport mechanisms activated when MRP2 is lacking. Drug interactions on these transporters may affect efficiency and toxicity of adefovir.

W-PO10006

ALTERED REGULATION OF WATER AND SODIUM TRANSPORT SYSTEMS IN THE KIDNEY OF RATS TREATED WITH GLYCYRRHIZIC ACID

KI CHUL CHOI[1], SEONG KWON MA[1], YOUN KYOUNG LEE[1], SOO WAN KIM[1], NAM HO KIM[1], JONGUN LEE[2]
Department of Internal Medicine, Chonnam National University Medical School[1], Department of Physiology, Chonnam National University Medical School[2]

The present study was aimed to determine whether there exists an altered regulation of aquaporin (AQP) water channels and sodium transport systems in the kidney in licorice-induced hypertension. Male Sprague-Dawley rats were treated with glycyrrhizic acid (GA) in drinking water (200 mg/dL) for 3 weeks. Systolic blood pressure (SBP) was measured by the tail-cuff method. Plasma renin activity (PRA), serum aldosterone levels, and plasma concentrations of arginine vasopressin (AVP) were determined by radioimmunoassay. The protein expressions of AQP1-3, heteromeric G protein subunit (Gsa) and Na,K-ATPase a1 subunit were determined in the kidneys by Western blot analysis. The enzymatic activity of Na,K-ATPase was also determined. SBP increased significantly following the GA treatment (129.7 ± 0.9 vs. 153.0 ± 4.1 mmHg, $p < 0.01$, $n = 10$ each). Accordingly, PRA was significantly decreased (3.7 ± 1.0 vs. 1.2 ± 0.6 ng/mL/hr, $p < 0.05$, $n = 6$ each). Serum aldosterone levels were also decreased significantly (19.6 ± 2.5 vs. 5.3 ± 0.8 ng/dL, $p < 0.05$, $n = 6$ each). Levels of plasma AVP did not differ between the groups. In the GA group, the expression of AQP1 was not significantly altered. The expression of AQP2 was significantly increased in the outer medulla and inner medulla, while unaltered in the cortex. The expression of AQP3 was also increased in the outer medulla and inner medulla, while unaltered in the cortex. The expression of Gsa was increased in the inner medulla, while unaltered in the cortex and outer medulla. The enzymatic activity of Na,K-ATPase was significantly increased in the kidney, and the protein expression of Na,K-ATPase a1 subunit was increased in the cortex of the kidney. The increased expression of AQP2–3 and the up-regulation of Na,K-ATPase may play a role in hypertension following the ingestion of glycyrrhizic acid.

W-PO10007

INFLUENCE OF ANTISENSE CUBILIN RNA ON ALBUMIN-INDUCED EXPRESSIONS OF CHEMOKINS IN HUMAN RENAL PROXIMAL TUBULAR EPITHELIAL CELLS (HK-2)

JURONG YANG[1], YU HY[1], LIN JING[1], HE YN[1]
Dept. of Nephrology, Daping Hospital of TMMU[1]

Objective: To investigate the role of cubilin on albumin-induced expressions of monocyte chemoattractant protein 1 (MCP-1) and regulated upon activation normal T-cell expressed and secreted (RANTES) in human renal proximal tubular epithelial cells (HK-2).
Methods: The sense and antisense cubilin recombinant eukaryotic expression vectors (pcDNA-CUB and pcDNA-ACUB) were constructed using gene recombining techniques and identified by restriction endonucleases digesting and DNA sequencing. Both vectors as well as pcDNA3.1(+) vector were respectively transfected into HK-2 cells with lipofectin DOTAP. Flow cytometer (FCM) was applied to determine the inhibitive effect of antisense cubilin RNA on albumin uptake of HK-2 cells. Posterior to cell transfection for 72 hours, high concentration of albumin (10 g/L) was used for stimulation for 24 hours. The expressions of MCP-1 and RANTES in HK-2 cells were measured by ELISA and Western blot.
Results: Compared with DOTAP group, the albumin uptake was significantly lowered ($p < 0.01$) and both MCP-1 and RANTES expressions of HK-2 cells significantly decreased in pcDNA-ACUB trasnfection group ($p < 0.001$).
Conclusion: Antisense cubilin RNA can inhibit albumin uptake of HK-2 cells and suppress the albumin-induced up-regulation of MCP-1 and RANTES expression in the HK-2 cells, as indicates that cubilin may play an important role in albumin-induced chemokines expression in renal proximal tubular epithelial cells.

W-PO10008

SYMPATHETIC REGULATION OF AQUAPORIN-2 WATER CHANNELS IN THE KIDNEY IN RATS

KI CHUL CHOI[1], YOONWHA OH[2], YOUN KYOUNG LEE[1], SEONG KWON MA[1], SOO WAN KIM[1], NAM HO KIM[1], JONGUN LEE[2]
Department of Internal Medicine, Chonnam National University Medical School[1], *Department of Physiology, Chonnam National University Medical School*[2]

Whether there exists a sympathetic neural mechanism in the regulation of aquaporin-2 (AQP2) water channels in the kidney was examined. Male Sprague-Dawley rats were used. Four different series of experiments were done. In the first series, the renal nerve was unilaterally denervated by stripping the nervous and connective tissues passing to and along the course of the renal artery and vein and painting these vessels with a solution of 10% phenol through a midline abdominal incision. They were kept for 3 days until sacrificed. In another series of experiment, chemical sympathectomy was achieved by intravenous injection of 6-hydroxydopamine (1 mg/kg). They were used 2 days later. In the third series, rats were unilaterally renal nerve-denervated and subjected to a water restriction. During the days of water restriction, the rats were intraperitoneally infused of V2 receptor antagonists (arginine vasopressin fragment 4–9, 5 μg/kg/day) through osmotic minipump. In the last series, deoxycorticosterone acetate (DOCA)-salt hypertension was induced in renal nerve-intact and denervated rats. The expression of AQP2 proteins was determined in the kidneys. Following the denervation, the norepinephrine content in the denervated kidney was significantly decreased, while that in the contralateral kidney increased. Accordingly, the expression of AQP2 proteins was markedly decreased in the denervated kidney. The chemical sympathectomy with 6-hydroxydopamine markedly decreased the expression of AQP2 proteins in the kidney. Following the water-restriction, the expression of AQP2 channels increased, however, the magnitude of which was lower in the denervated than in the contralateral innervated kidney. The renal nerve denervation significantly attenuated the development of DOCA-salt hypertension, along with a less increased expression of AQP2 proteins in the kidney. It is suggested that the sympathetic nervous system has a tonic stimulatory effect on the expression of AQP2 water channels in the kidney.

W-PO10009

EFFECTS OF FUROSEMIDE ON GENE EXPRESSION OF RENAL CALCIUM AND MAGNESIUM TRANSPORT MOLECULES

CHIEN-TE LEE[1], HUNG-CHUN CHEN[2], KIM-CHONG YONG[3], LI-WEN LAI[3], YEOUNG-HAU LIEN[3]
Chang-Gung Memorial Hospital, Kaohsiung, Taiwan[1], *Graduate Institute of Medicine, Kaohsiung Medical University*[2], *Department of Medicine, Health Sciences Center, University of Arizona, USA*[3]

Loop diuretics are effective agents in treating edema and hypertension. In addition to diuresis and natriuresis, increased urinary calcium (Ca) and magnesium (Mg) excretion are often observed. The pathomechanism of the enhanced Ca and Mg excretion is direct inhibition on reabsorption in thick ascending limb of Henle. As the distal convoluted tubule (DCT) plays an important role in Ca and Mg transport, we aim to evaluate the alternations in renal Ca and Mg transport in DCT after furosemide treatment. In acute experiments, the mice were administered with single dose furosemide (10 mg/kg/dose), and for chronic experiments, twice injections with same dosage were given for 3 days. Salt drinking water was given as supplement to prevent diuretics-induced volume depletion in chronic experiment. Serum creatinine (Cr), Ca and Mg were determined as well as urine samples. Quantitative analysis of gene expression was performed using real-time RT-PCR. Our results showed that urinary Ca and Mg excretion were increased after single injection (urinary Ca/Cr: 0.09 ± 0.11 *vs.* 0.18 ± 0.02, Mg/Cr: 0.48 ± 0.14 *vs.* 1.31 ± 0.27, both $p < 0.05$). Significant increase in gene expression of TRPV5 ($174 \pm 10\%$ of control) and calbindin-D28k ($222 \pm 23\%$) were found. Chronic treatment also induced calciuresis and magnesiuria with or without salt supplement (Ca/Cr: 0.26 ± 0.17, 0.32 ± 0.14; Mg/Cr: 0.96 ± 0.1, 0.98 ± 0.1, all $p < 0.05$). This effect was associated with increased expression of Ca and Mg channels and calbindins (TRPV5: $170 \pm 10\%$, $212 \pm 15\%$; TRPM6: $230 \pm 11\%$, $293 \pm 19\%$; calbindin-D28k: $310 \pm 18\%$, $290 \pm 21\%$; calbindin-D9k: $220 \pm 20\%$, $255 \pm 18\%$, all $p < 0.05$). We conclude that furosemide treatment causes significant renal Ca and Mg loss. Salt supplement did not correct the renal wasting. Up-regulation of Ca and Mg transport molecules in DCT represent an adaptive response to increased Ca and Mg delivery induced by furosemide.

W-PO10010

ANION TRANSPORTERS SLC26A6 AND SLC26A7 HAVE SPECIFIC EXPRESSION IN JUXTAGLOMERULAR APPARATUS OF HUMAN KIDNEY

MINNA KUJALA[1], JUKKA TIENARI[2], HANNES LOHI[3], HANNU SARIOLA[4], EERO LEHTONEN[5], JUHA KERE[6]
Department of Medical Genetics, University of Helsinki, and Department of Urology, Helsinki University Central Hospital, Finland[1], *Department of Pathology, Helsinki University Central Hospital/Peijas Hospital, Finland*[2], *Department of Medical Genetics, University of Helsinki, Finland*[3], *Institute of Biomedicine, Developmental Biology, University of Helsinki, and HUCH Laboratory Diagnostics, Helsinki University Hospital, Finland*[4], *Department of Pathology, Haartman Institute and Helsinki University Central Hospital, University of Helsinki, Finland, and Department of Cellular and Molecular Medicine, University of California at San Diego, La Jolla, CA, USA*[5], *Department of Medical Genetics, University of Helsinki, Finland, and Department of Biosciences at Novum, Karolinska Institutet, Stockholm, Sweden*[6]

Background: The solute carrier protein family 26 (SLC26), formerly known as sulphate transporters, consists of membrane proteins all of which but one transport anions with different specificities. Two of them, SLC26A6 (PAT1) and SLC26A7, are expressed in kidney, but their exact localization in human kidney has not been reported. Both proteins transport at least chloride, oxalate, sulphate and bicarbonate. Interestingly, expression and function of at least some of the orthologous SLC26 proteins vary between different mammalian species. We therefore studied expression of SLC26A6 and A7 in human kidney.
Methods: Localization of SLC26A6 and A7 in human kidney was studied by RT-PCR, Western blotting and immunohistochemistry.
Results: In addition to expression in specific tubule segments, both SLC26A6 and A7 are localized to the juxtaglomerular apparatus. SLC26A6 is expressed in the macula densa (MD) cells, while SLC26A7 is localized to the extraglomerular mesangial cells (EMC) in human kidney. To our knowledge, expression of these anion transporters has never before been reported in the juxtaglomerular apparatus of any species.
Discussion: Both MD and EMC are important in the tubuloglomerular feedback (TGF) involved in the autoregulation of glomerular filtration and renal blood flow. MD functions as the sensor of the luminal $Na+$ and $Cl-$ concentrations.

Concentration alterations trigger signals from MD via EMC to the smooth muscle cells of the afferent arteriole. Cl- is thought to have an important role especially in the first steps of this cascade. Thus MD and EMC expression of the anion transporters SLC26A6 and A7, capable of transporting e.g. chloride, fit well to the previous functional knowledge and suggest that SLC26A6 and A7 could have roles in TGF. Functional studies of these anion transporters in TGF are warranted.

W-PO10011

GLOBAL CALCIUM OSCILLATIONS INDUCED GLOMERULAR MESANGIAL CONTRACTION AND THE MECHANISM OF ITS DEVELOPMENT

ZHE FENG[1], XIANGMEI CHEN[1], CHAOLIANG WEI[1], HEPING CHENG[1], JIANZHONG WANG[1]
General Hospital of PLA[1]

Backgroud: The contraction of GMCs could regulate glomerular capillary surface area and the filtration rate. In GMCs, increasing calcium induced contraction, but it was unknown whether calcium oscillations were linked to the contraction, and nor was its mechanism of development. In this study, we investigated the mechanism and the relationship between cellular contraction and calcium oscillations.
Methods: primary passage cultured GMCs were used for experiments, which were loaded with fluo-3 to observe the rising form of calcium using confocal microscopy. In addition, to illuminate the mechanism of its development, the cells were observed after stimulation with 10nM angiotensin II, nicardipine, 2-APB, TG, Ryanodine, Et-18-OCH3, and NFA, respectively. We also explored the relationship between calcium events and contraction using Mesangial Cell Planar Area Measurements and the analysis of MLC20 phosphorylation. The important roles of IP_3 and ryanodine receptor subtypes in the process of contraction of GMCs were disclosed using immunofluorescence co-localization assay.
Results: We demonstrated two forms of calcium events: local calcium waves and global calcium oscillations. The calcium events were all inhibited by nicardipine, 2-APB, TG, ET – 18 – OCH_3, but not by ryanodine. The oscillations were only inhibited by NFA, but the waves not. It was also found that oscillations could initiate the contraction, but waves not. GMCs expressed colocalized ryanodine type 1/3 and IP_3 type 1 receptors. These data showed that transiently-rising calcium level from the ryanodine and IP_3 receptors couple could induce the glomerular mesangial cell contraction.
Conclusion: Global calcium oscillations induced the GMCs' contraction through the Ca^{2+} releasing IP_3 receptor and succedent Ca^{2+}-induced ryanodine receptor coupling.

W-PO10012

INCREASED U-AQUAPORIN-2 IN RESPONSE TO LOOP DIURETIC TREATMENT IN PATIENTS WITH CHRONIC HEART FAILURE

JESPER N BECH[1], JØRN STARKLINT[1], OLE NYVAD[1], PREBEN JENSEN[1], ERLING B PEDERSEN[1]
Dept. of Medicine, Holstebro Hospital, Denmark[1]

Background: patients with chronic heart failure (CHF) have decreased ability to excrete water and have increased urinary excretion of aquaporin 2 (U-AQP2). The natriuretic and diuretic effects of furosemide are antagonized by an increased reabsorption of sodium and water in the collecting ducts. It is unknown if aquaporin-2 (AQP2) renal water channels are involved in this compensatory reabsorption. We tested the hypothesis that U-AQP2 increases after a single intravenous dose of furosemide in CHF patients.
Methods: In a randomized, single blind, placebo controlled, cross over study, we measured the effect of furosemide, 80 mg, on U-AQP2, urine volume, free water clearance (C_{H_2O}), and fractional excretion of sodium (FE_{Na}) in twelve CHF patients. Plasma concentrations of vasopressin (AVP), renin (PRC), angiotensin II (Ang II), aldosterone (Aldo), atrial (ANP) and brain natriuretic peptides (BNP) were measured during the study. U-AQP2 and hormones were determined by radioimmunoassays.
Results: Furosemide increased U-AQP2 (140%), urine volume (280%), C_{H_2O} (95%), and FE_{Na} with a factor of 15 ($P < 0.008$ for all). Furosemide increased AVP (51%), PRC, Ang II (86%), and Aldo (59%), ($P < 0.021$ for all). ANP and BNP did not change. After placebo Aldo decreased 18% ($P < 0.023$), and the other variables were unchanged after placebo.
Conclusions: In CHF patients furosemide increased U-AQP2 indicating increased water reabsorption in the distal part of the nephron. This is most likely a compensatory phenomenon in addition to increased activity of the renin-angiotensin-aldosterone system, to prevent excess loss of sodium and water.

W-PO10013

A MULTIDEFICIENT DIET IMPAIRS RENAL H20 AND NA+CONSERVATION BUT INCREASES GLOMERULAR AND PROXIMAL TUBULAR FUNCTION

CARMEN DE CASTRO CHAVES[1], JOAO HENRIQUE DA COSTA SILVA[2], ELISA SUEMITSU HIGA[3]
Fisiologia e Farmacologia, Universidade Federal de Pernambuco-UFPE, Brasil[1]; Fisiologia e Farmacologia, Universidade Federal de Pernambuco-UFPE, Brasil[2], Nefrologia, Universidade Federal de São Paulo EPM-UNIFESP, Brasil[3]

Malnutrition is still significant in southern Pernambuco State (Brazil). As protein deficiency is involved in hypertension and the kidney controls blood pressure, the effects of a protein-aminoacid deficient diet (DD) were evaluated on water balance, Na^+ and K^+ excretions, glomerular (GFR) and proximal tubular (PTF) functions.
Methods: 24 rats were fed commercial diet (CD, n = 12) or the DD (n = 12) from weanling and monitored weekly at growth (7–11th week) and adult (17–19th week) ages in metabolic cages for 24 h while GFR and PTF were assessed by 3 h creatinine and Li^+ clearances at 20th week. Results, in mean ± SD, were significant when $p < 0.05$ (Student's 't' test).
Results: The similar weight (g) at weanling (40.3 ± 3.8 vs 40.3 ± 2.8; 98.4 %) decreased progressively on DD rats being 34.9 % of CD at adult (427.3 ± 32.6 vs 144 ± 15). With a similar water ingestion (mL/100 g), urine volume (mL/100 g) was higher in DD rats from the 9–11th weeks and in adults with lower specific gravities (g/ml) at all weeks. DD rats Na^+ excretion (μEq/24 h/100 g) was higher from 8th- to 11th weeks and in adults (71.7 ± 29.5 vs 295.7 ± 64.1) while K^+ excretion (μEq/24 h/100 g) was higher at the 11th week and in adults (146.4 ± 24.5 vs 246.6 ± 55.5). Creatinine and Li^+ clearances (μl/min/100 g; n = 8) increased in DD rats, respectively: 259.3 ± 64.0 vs 374.8 ± 124.0 and 15.7 ± 16.7 vs 68.3 ± 30.9. Blood and urine nitric oxide (μM) did not differ between CD and DD rats.
Conclusions: The ingestion of a multideficient diet from weanling impairs weight gain, the renal ability to conserve H_2O and Na^+ at all ages, increasing GFR and PTR without disruption of the glomerular-tubular balance in adult rats.
Support: CNPq/PROPESQ and Laboratório Paulo Loureiro.

Acid-Base & Electrolyte Disorder

W-PO10014

DISTAL RENAL TUBULAR ACIDOSIS-SPECTRUM OF CLINICAL PRESENTATION AND OUTCOME

RATAN JHA[1], JAGADISH KUMAR[1], GIRISH NARAYAN[1], NIYAZ AHMED[1], RAMESH CHANDRA[1]
Medwin Hospital[1]

Introduction: Distal renal tubular acidosis (d RTA) often presents with non-specific manifestations. The aim of this study is to present our 10 years experience (1994–2004)of d RTA (type I) with reference to clinical characteristics and outcome.
Methods: 76 patients (42 male, 34 female, age 3–75 yrs) of dRTA with a median follow up of 3 years (1–10 yrs) were analyzed. Haemogram, urine pH, ABG, renal function, electrolytes, calcium, phosphorus, LFT, ultrasound of abdomen, autoimmune profile, MCU, skeletal survey, i PTH, urine anion gap ammonium chloride load test (if HCO3 > 15) were done. For comparison of renal outcome patients were categorised in to two groups based on calculated GFR (Group A above 60,Group B below 60). Patients of renal transplant, and significant renal impairment (GFR <30 ml) were excluded.
Results: Diagnosis of dRTA got delayed by 1–10 yrs. Presentations were bone pain (11), paralysis/walking difficulty (28), voiding disturbance (21), failure to thrive (16). Radiological investigation showed osteomalacia or rickets (26), renal stone (10) or nephrocalcinosis (7). Biochemical abnormalities were hypokaelemia (30), hyperkalemia (06), hypercalcimia (03), and hyperchloraemic metabolic acidosis (71). The secondary causes of distal RTA were apparent in 40 patients, the commonest being urological causes (24) (others congenital syndrome (02), autoimmune (03), primary hyperparathyroidism (03), medullary sponge kidney (03), flourosis (05)). Treatment included sodium bicarbonate, potassium citrate, calcium supplements, spironolactone, calcitriol and corrective surgery, which were individualized. Renal dysfunction worsened in 14 patients (1 in-group A, 13 in-group B).

Conclusion: Most patients experienced improvement of symptoms. Children with dRTA had high incidence of urological abnormality, which persisted despite corrective surgery. Unlike other causes of dRTA, primary hyperparathyroidism surgery resulted in cure.

W-PO10015

GROWTH VELOCITY IN CHILDREN WITH PRIMARY DISTAL TYPE RENAL TUBULAR ACIDOSIS

AN-YI LIN, MD[1], CHING-YUAN LIN, MD, PHD[1]
Changhua Christian Children's Hospital[1]

Primary distal type tubular acidosis (RTA-type 1) results from defects of distal renal tubules in urinary acidification. In an attempt to characterize the clinical features, growth and outcome of primary distal type RTA in Taiwan, we retrospectively studied 28 patients (16 males and 12 females) of primary distal type RTA in our hospital in the past 13 years. The mean age at diagnosis is 2 years and 6.8 months. Hematuria is noted in 5 of 25 cases (20%). Nephrocalcinosis is noted in 5 of 21 cases (23.8%). The mean value of Uc_a/Ucr is 0.313 ± 0.067 in those older than 2 years of age and 0.262 ± 0.152 in those younger. Rickets is suspected in only one child by radiologic study. At initial diagnosis, the mean bone age is delayed for 16 months. The older the patient is, the more the bone age delayed. The mean value of height is −2.81 standard deviation score (SDS); the mean value of body weight is −2.44 SDS; and the mean value of growth velocity in height is −2.62 SDS. After alkali therapy given for a duration from 8.5 months to 5 years and 7 months, improvement is noted in height up to −1.69 SDS, in body weight up to −1.10 SDS, and in height growth velocity up to 1.06 SDS respectively.

W-PO10016

ROLE OF PLASMA FIBROBLAST GROWTH FACTOR-23 IN ACIDOSIS-INDUCED HYPOPHOSPHATEMIA

RATCHANEE CHEAWCHANTHANAKIJ[1], WASANA STITCHANTRAKUL[2], SINEE DISTHABANCHONG[3], SOMNUEK DOMRONGKITCHAIPORN[1]
Nephrology Medicine, Ramathibodi Hospital, Thailand [1]*, Reserch Center, Medicine, Ramathibodi Hospital, Thailand*[2]

Background: Fibroblast growth factor-23 (FGF-23) has an inhibitory effect on renal tubular phosphate reabsorption. Renal phosphate wasting is a common finding in patients with distal renal tubular acidosis (dRTA) and during acid loading in normal subjects. We conducted a study to determine the role of FGF-23 during acid loading in normal subjects and in patients with dRTA.
Methods: Ten healthy volunteers who were given NH4Cl for seven days and five dRTA patients who received potassium citrate for three months were enrolled. Serum and urine parameters including iPTH and FGF-23 were collected at baseline and at the end of seven days in normal subjects and after three months in patients with dRTA.
Results: After a seven day-course NH4Cl ingestion, serum bicarbonate 26.3 (23–32) *vs* 19.4 (14–22) mmol/L, inorganic phosphate 1.23 (0.97–1.62) *vs* 0.97 (0.65–1.29) mmol/L and calcium 2.3 (2.18–2.5) *vs* 2.18 (2.03–2.45) mmol/L, decreased significantly from baseline, which coincided with an increase in fractional excretion of calcium 1.75 (0.70–2.74) *vs* 3.97 (1.11–7.39) % and phosphate 8.13 (5.98–13.9) *vs* 13.40 (9.30–20.10) %, $p < 0.05$. Plasma levels of FGF-23 10.89 (3.86–92.36) *vs* 6.70 (1.36–60.13) pg/ml and iPTH 36.17 (24.6–55.5) *vs* 23.98 (13.9–40.9) pg/ml were suppressed during ammonium chloride-induced metabolic acidosis ($p < 0.05$). There was no significant correlation between serum calcium, phosphorus, bicarbonate or iPTH and FGF-23. Among dRTA patients, serum bicarbonate rose after correction of metabolic acidosis with potassium citrate. Similar to the healthy volunteers, a tendency toward low iPTH was observed during metabolic acidosis. Serum phosphate and FGF-23 levels were not significantly altered before and after treatment of potassium citrate.
Conclusions: It is unlikely that FGF-23 is responsible for the renal phosphate wasting observed during chronic metabolic acidosis.

W-PO10017

FLUOXETINE-STIMULATED WATER ABSORPTION IN NORMAL RAT IMCD CAN ACCOUNT FOR THE ELDERLY HYPONATREMIA

ZENAIDE-P MOYSES[1], FAUSTO K NAKANDAKARI[1], ANTONIO J MAGALDI[1]
Clinical Hospital-University of São Paulo-Nephrology LIM[1]

Fluoxetine, is currently used in depressant elderly. However, hyponatremia have been reported in the treated with this drug. The pathogenesis of its effect is not well known but this hyponatremia has been attributed to a syndrome of inappropriate antidiuretic hormone secretion caused by Fx, although the vasopressin enhanced plasma level has not been well demonstrated yet. Thus, this experiment was designed to investigate if Fx has a direct effect in the IMCD from normal rats. Two kinds of experiments were done: 1) 'in vivo?study- 10 rats were supplied daily with i.p. injections of Fx 10 mg/kg. Previously to the Fx use, the Na^+ and K^+ blood level was dosed as a control period. Five days after that, the rats were sacrificed and blood was collected. 2) 'in vitro?study – The osmotic water permeability ($Pf \times mm.s^{-1}$), was determined at 37C and pH 7.4,in normal rats IMCD ($n = 6$) perfused and bathed with Ringer/HCO_3. Fx (10^{-7} M) was added to the bath fluid. The results from the 'in vivo?study showed that the weight decreased from 179?.4 g to 158?.0 g($p < 0.001$); the urinary volume decreased from 14,5?.5 ml/12 h to 7.5?.7 ml/12 h($p < 0.01$); the Na^+ plasma level decreased from 140.0?.78mEq/l to 134.7?.5 mEq/l ($p < 0.01$) and the K^+ and the ADH plasma level remained unchanged. The data showed that Fx increased the Pf in IMCD in absence of vasopressin from the control period- 22.6?0.0 × $mm.s^{-1}$ to Fx- 55.9?8.9 × $mm.s^{-1}$ ($p < 0.05$). In summary our data revealed that after Fx supply, weight, urinary volume, and plasma Na^+ level decreased significantly and the plasma ADH level remained unchanged, whereas the water absorption in IMCD increased, leading us to conclude that the direct effect of Fx in the IMCD, increasing Pf in absence of vasopressin, can account for the hyponatremia produced by this drug in depressant elderly.

W-PO10018

POST TRAUMATIC NEPHROGENIC DIABETES INSIPIDUS

PARLINDUNGAN SIREGAR[1], AIDA LYDIA[1], EKA J. WAHJOEPRAMONO[2]
Division of Nephrology and Hypertension, Department of Internal Medicine, University of Indonesia, Jakarta[1]*, Department of Neuro-Surgery, Siloam-Gleneagles Hospital, Lippo-Karawaci, Tangerang, Indonesia*[2]

Central Diabetes Insipidus (DI) is well known to occur after head injury. It is common to suspect impaired vasopressin synthesis and/or release mechanisms and to diagnose Central DI when water diuresis ensues after head injury. We present three cases with polyuria which were hospitalized due to severe traffic accidents that paradoxically resulted in the diagnosis of Nephrogenic DI. Y, a 17-year-old male with subdural and epidural hemorrhage, operated on admission, died at day 14^{th}. Polyuria started at day 10^{th}. Plasma sodium 170 mmol/L; 24 hours urine electrolytes : Na 908, K 45, Cl 1991 mmol/L. V, 17-year-old female with right intracerebral, right subdural and subarachnoid hemorrhage, and right temporal pneumocephalus, was operated on admission. Polyuria lasted from day 12^{th} until day 27^{th}. Plasma sodium was normal; 24 hours' urine electrolytes: Na 855, K 48, Cl 966 mmol/L. Urine osmolality 302 mosm/Kg. No response to Desmopressin 30 ugr/day intranasally.S, 50-year-old male with Cerebral Concussion, right femoral fracture and fracture of the eight right costae. Polyuria lasted from day 5^{th} until day 26^{th}. Plasma sodium was normal; 24 hours' urine electrolytes : Na 1288, K 83, Cl 554 mmol/L. Urine osmolality was 149 mosmol/Kg. No response to Desmopressin 60 ug/day. Due to the worse condition we did not perform the Water Restriction test. Two of our patients (patients S and V) were resistant to Desmopressin and the urine volume returned to normal without Desmopressin. Our patients had high urine excretion of Na and K showing the defect of the tubular reabsorbtion. It seems that there was an anti vasopressin or vasopressinase in our severe head injury traffic accident patients. In conclusion, polyuria in our patients who were resistant to Desmopressin with high excretion of Na and Cl, were suggestive of being caused by Nephrogenic Diabetes Insipidus.

W-PO10019

SERUM IONIZED MAGNESIUM IN DIALYSIS PATIENTS

GHEUN-HO KIM[1], CHANG HWA LEE[1], CHONG MYUNG KANG[1], YOUNGSOON KIM[2], JANG WON SEO[2]
Hanyang University College of Medicine[1], Hallym University Hangang Sacred Heart Hospital[2]

Magnesium exists in serum in three fractions, that is, one protein-bound and two ultrafilterable fractions consisting of complex-bound and free ionized magnesium (iMg). However, only the fraction of iMg is biologically active. To evaluate fractional changes in the serum magnesium in dialysis patients we measured serum iMg in 29 hemodialysis (HD) patients and 23 peritoneal dialysis (CAPD) patients using an ion-selective electrode. The results were compared with those from 36 age-matched control patients, who were admitted due to nonrenal diseases during the same study period and were without any metabolic disorders. Serum total magnesium (tMg) was significantly higher in both HD (0.96 ± 0.03 mmol/L) and CAPD (0.89 ± 0.04 mmol/L) patients when compared with controls (0.73 ± 0.02 mmol/L). Serum iMg was significantly higher in HD patients (0.66 ± 0.02 mmol/L) but not in CAPD patients (0.56 ± 0.02 mmol/L), compared with controls (0.54 ± 0.01 mmol/L). Thus, the ionized fraction of serum magnesium was significantly decreased in CAPD patients ($63.8 \pm 1.4\%$) but not in HD patients ($70.6 \pm 1.9\%$), compared with controls ($75.3 \pm 2.0\%$). Although tMg correlated significantly with iMg ($r = 0.69$, $P < 0.001$), neither tMg nor iMg correlated with serum albumin. Serum albumin was significantly lower in CAPD patients (3.42 ± 0.11 g/dL) but not in HD patients (3.71 ± 0.08 g/dL), compared with controls (3.80 ± 0.12 g/dL). These data suggest that true hypermagnesemia may be a characteristic in HD patients (at predialysis) but not in CAPD patients based on the serum iMg measurement and that the ionized fraction of serum magnesium may be decreased in CAPD patients but not in HD patients. It is concluded that it may be worthwhile measuring serum iMg in dialysis patients to detect disturbances in magnesium homeostasis. Mechanisms to produce fractional changes in serum magnesium remain to be elucidated.

W-PO10020

CYCLOSPORINE A ATTENUATES CYCLOOXYGENASE-2 EXPRESSION AND PROSTAGLANDIN E2 RELEASE DEPENDED ON NUCLEAR FACTOR AT

DONGYAN LIU[1]
Peking Union Medical College Hospital, China[1]

Background: Macula densa cells are specialized tubular epithelial cells that play a central role in NaCl dependent control of glomerula arteriolar tone and rennin release. In high rennin states, cyclooxygenase-2(COX-2) expression in MD region is increased. The decreasing of GFR is one of the adverse effects of Cyclosporine A (CsA). The COX-2 gene promoter contains functional binding sites for the nuclear factor of activated T cells (NFAT) has been confiemed recently. The purpose of our study is to assess the effect of CsA on COX-2 expression in the kidney and whether NFAT was involved.
Method: Male Sprague-Dawley rats with low salt diet were treated with CsA (15 mg/kg/d), celecoxib (40 mg/kg/d). In low salt media,COX-2 protein expression in mouse macula densa cells (MMDD1) was tested in the absence and in the presence of CsA. The NFAT protein expression and luciferase activity of COX-2 promoter in low NaCl and normal NaCl was tested.
Results: CsA, celecoxib lowered the low salt diet induced the increasing excretion of prostaglandin E_2 (PGE_2);sodium,water was lowered ($P < 0.05$). Basal and low salt induced stimulation of plasma renin activity (PRA) and of renal cortex renin mRNA was further enhanced by CsA. By contrast, celecoxib attenuated the low salt induced rise of PRA and of renin mRNA, both in the absence and in the presence of CsA ($P < 0.01$). CsA inhibited the stimulation of COX-2 protein expression by low salt media ($P < 0.01$). NFAT luciferase activity ($P < 0.05$) and protein expression increased by low salt media ($P < 0.01$).
Conclusion: It suggested that the expression of COX-2 in the kidney could be inhibited by CsA; an NFAT cis-elementmay may located in COX-2 promoter and may be stimulated by low salt media. The inhibition of COX-2 may therefore in part accout for the known adverse effects of CsA in the kidney.

THEME 2: PROGRESSION AND PREVENTION OF CHRONIC RENAL FAILURE

Pathogenesis Of Progression – Angiotensin

W-PO20001

MODIFICATION OF DELAYED RADIATION NEPHROPATHY FOLLOWING INTERNAL IRRADIATION WITH ALPHA PARTICLES

JASPREET SINGH JAGGI[1], SURYA V. SESHAN[2], MICHAEL R. MCDEVITT[1], DAVID A. SCHEINBERG[1]
Memorial Sloan Kettering Cancer Center, New York, USA[1]; Weill Medical College of Cornell University, New York, U.S.A.[2]

Background: Radiolabeled monoclonal antibodies have shown enormous potential as targeted anti-cancer agents in a variety of clinical and pre-clinical studies. However, the development of delayed radiation nephropathy is a possible hurdle to the safe therapeutic application of these agents.
Design: We attempted to modify pharmacologically, the functional and morphological changes in mouse kidneys after injection with an Actinium (^{225}Ac) nanogenerator, a molecular-sized, antibody-targeted, *in vivo* generator of alpha particle emitting elements. Animals were injected with 0.35 μCi of ^{225}Ac-labeled antibody, a dose sufficient to produce delayed radiation nephropathy, and then randomized to different treatment groups. Captopril, an ACE inhibitor and l-158809, an Angiotensin II receptor-1 blocker, were administered continuously in drinking water at 500 mg/l and 25 mg/l respectively. Spironolactone (30 mg/kg/day) was administered as a sustained-release pellet, implanted every 3 months. The modifying treatments were started 10 days after radioimmunoconjugate injection (to prevent alteration of radioimmunoconjugate pharmacokinetics) and continued until the animals were sacrificed (40 weeks post injection).
Results: Untreated control mice showed a significant increase in the BUN (92.5 ± 6.3 mg/dl). Their renal histopathology revealed subcapsular cortical atrophy, dilated Bowman spaces, tubulolysis with collapse, tubular basement membrane thickening and some regenerated, simplified tubules. Captopril treatment accentuated the functional ($BUN = 130.5 \pm 11.4$ mg/dl; $p < 0.05$ vs. control) and histopathological damage, whereas l-158809 offered moderate protection ($BUN = 61.7 \pm 7.7$ mg/dl; $p = 0.02$ vs. control). However, spironolactone treatment significantly prevented the development of histopathological and functional changes ($BUN = 34 \pm 7.1$ mg/dl; $p < 0.001$ vs. control). TGF-β1 expression in preserved tubular cells was higher in captopril treated animals and lower in animals treated with l-158809 or spironolactone, as compared to controls.
Conclusion: Low-dose spironolactone and l-158809 to a lesser extent may be used to protect against the potential nephrotoxicity of ^{225}Ac labeled immunoconjugates.

W-PO20002

HIGHER PLASMA ALDOSTERONE CONCENTRATION IS A RISK FACTOR FOR PROGRESSION OF RENAL INJURY IN HYPERTENSION

HIDETSUNA WATANABE[1], TETSUO KATOH[2], TSUYOSHI WATANABE[2], PEDRO A. JOSE[3]
Health Care Center, Fukushima University[1], The Third Department of Internal Medicine, Fukushima Medical University, Fukushima, Japan[2], Georgetown University Medical Center, Washington, DC, USA[3]

Renin/angiotensin II and aldosterone-induced renal injury are associated with perivascular inflammation, cell proliferation, and increased superoxide production. The relationship between plasma renin activity (PRA), plasma aldosterone concentration (PAC), and progression of renal disease has not been established. To address this issue, we prospectively (15 years) investigated the association among PRA, PAC, and renal function in subjects with hypertension. Subjects with diabetes, proteinuria, hematuria, and serum creatinine >1.2 mg/dl were excluded. At the beginning of the study, PRA and PAC were not different between hypertensive ($N = 81$) and normotensive ($N = 140$) controls, suggesting that increased PRA and PAC, per se, are not major risk factors for hypertension. We compared patients whose serum creatinine increased by 50 % or greater (progressors, n = 11) and those who did not (nonprogressors, n = 70) over a 15 year period. At baseline, sex distribution, age, duration of hypertension, initial antihypertensive therapy, serum creatinine, incidence of microalbuminuria, and blood sugar were similar in progressors and nonprogressors. However, the progressors had higher baseline PRA and PAC levels than those noted in nonprogressors; PRA and PAC levels positively correlated with the incidence of increased (≥50%) serum creatinine (P = 0.001). Relative to the group with lowest quartile PRA and PAC, the odds ratios for each of the groups with progressively higher were 0.86, 3.9, and 9.2 for PRA and 1.0, 2.0, and 7.1 for PAC. For those with hypertension, elevated PRA and PAC levels at the initial visit were predictive of progression of renal dysfunction (≥50% rise in serum creatinine); subjects with PRA levels ≥2.0 ng/ml/hr had an odds ratio of 5.2 and a confidence interval of 1.6–17.4; PAC levels ≥140 pg/ml had an odds ratio of 5.3 and a confidence interval of 1.5–18.2. PRA ≥ 2.0 ng/ml/h and PAC ≥140 pg/ml may be useful predictors of the long-term adverse renal functional consequences of hypertension.

W-PO20003

ADDITIVE EFFECT OF ULTRA HIGH DOSE (64 MG) CANDESARTAN ON PROTEINURIA: A DOUBLE BLIND RANDOMIZED STUDY

ROLAND E. SCHMIEDER[1], ARNFRIED U. KLINGBEIL[1], ERWIN H. FLEISCHMANN[1], CHRISTIAN DELLES[1], ROLAND VEELKEN[1]
University Erlangen-Nuremberg, Department of Medicine/Nephrology[1]

Both ACE inhibitors (ACE-I) and AT1 receptor blockers (ARB) effectively reduce proteinuria given in standard dose. We analyzed the antiproteinuric effect of candesartan (cand) at ultra high dosis in a randomized, double-blind forced titration study.

Hypertensive and normotensive patients with normal or mildly impaired renal function (creatinine clearance >30 ml/min) and a protein excretion rate of 1–10 g/day and a treatment with an ACE-I or ARB were eligible. If renal function and protein excretion rate were stable for 2 month, the trial started with a 4 week treatment of cand 16 mg. Thereafter, patients were randomly assigned to double-blind therapy with cand 32 mg (15 patients) or cand 64 mg (17 patients) for 12 weeks, followed again by a 4 week treatment of cand 16 mg.

Protein excretion after the first candesartan 16 mg phase was identical prior to randomisation (cand 32 mg: 2.7 ± 2.1 g/day; cand 64 mg 2.7 ± 1.3 g/day, n.s.). After 3 months protein excretion was reduced to 1.9 ± 1.2 g/day in the 64 mg group ($p < 0.017$) but not in the 32 mg group (2.8 ± 2.6 g/day), with a difference in the change of proteinuria between the two groups ($p < 0.025$). After down titrating to cand 16 mg protein excretion increased to 3.0 ± 1.7 g/day in the 64 mg group ($p < 0.022$), and remained unchanged in the 32 mg group (3.1 ± 3.0 g/day, n.s.). No change in creatinine clearance and 24-h ambulatory blood pressure was noticed in response to the different doses of candesartan in and between the two groups.

Our data indicate an additive antiproteinuric effect of ultra high dose treatment with the AT1 receptor blocker candesartan 64 mg compared to a standard standard dose.

W-PO20004

LIPID PEROXIDATION IN RENAL ISCHEMIC REPERFUSION: EFFECT OF ANGIOTENSIN INHIBITION

YUYEN SEUJANGE[1], WIPAWEE KITTIKOWIT[2], SOMCHAI EIAM-ONG[3], SOMCHIT EIAM-ONG[4]
Inter-department of Physiology, Graduate School, Chulalongkorn University, Bangkok 10330, Thailand[1]*, Department of Pathology, Faculty of Medicine, Chulalongkorn University*[2]*, Department of Medicine, Faculty of Medicine, Chulalongkorn University*[3]*, Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand*[4]

During renal ischemic reperfusion (IR), both reactive oxygen species (ROS) and angiotensin II (ANG II) are increased and play an important role in renal injury. ANG II increases ROS, especially superoxide (O2·), through activating membrane associated NADH/NAD(P)H oxidase activity. ROS can produce cellular injury by attacking membranes through the peroxidation of polyunsaturated fatty acids. Lipid peroxidation (LPO) of mitochondrial, lysosomal and plasma membrane could alter both membrane structure and function. The present study was conducted to investigate the role of angiotensin inhibition on LPO and nephropathy in IR. The male Wistar rats were subjected to 15-, 30-, 45- or 60-minute left renal artery occlusion (I). In 30-minute I group, the additional animals were followed by reperfusion (R) for 1 day (I30R1) in which were treated with water, or angiotensin converting enzyme inhibitor (ACEI; enalapril 5 mg/kg/d), or angiotensin receptor type 1 antagonist (ARA; losartan 10 mg/kg/d). Renal tissue malondialdehyde (MDA), a secondary product of oxidative stress formed during LPO was examined. Renal pathology also was determined. The findings showed that renal tissue MDA levels were progressively increased with increasing time of ischemia and was maximum at 30 minute of ischemia ($p < 0.01$). One day after reperfusion, the MDA level was still high ($p < 0.01$) as compared to control. Both ACEI and ARA could attenuate the heightened MDA levels ($p < 0.01$). Histological changes in 30-minute I group were slightly tubular dilatation and congestion. The progression of renal pathology was observed after 1 day of reperfusion. IR-induced nephropathy was ameliorated by ACEI or ARA administration. These results indicate that the inhibition of angiotensin could diminish lipid peroxidation and nephropathy during IR condition.

W-PO20005

POSSIBLE ROLE FOR MAST CELL CHYMASE IN THE PATHOGENESIS OF TUBULOINTERSTITIAL DAMAGE IN GLOMERULONEPHRITIS

YOSHIO KONISHI[1], TAKASHI MORIKAWA[2], NORIYUKI OKADA[3], CHIZUKO KITABAYASHI, KATUNOBU YOSHIOKA, MICHIAKI OKUMURA, MIZUO MIYAZAKI, MAKIKO UEDA, MASAHITO IMANISHI
Internal Medicine, Osaka City General Hospital, Japan[1]*, Department of Pharmacology Osaka Medical College*[2]*, Department of Pathology Osaka City University*[3]

Background: Mast cell chymase has been shown to deteriorate the renal tubulointerstitial damage, i.e. cell infiltration and fibrosis. One cause of the tubulointerstitial fibrosis is considered to be chronic ischemia as a consequence of peritubular capillary injury, while the mast cells have been reported to be induced by ischemia. Here, in glomerulonephritis, we investigated the relations among the mast cell chymase, the tubulointerstitial damage, and the chronic ischemia that is disorder of renal circulation.

Methods: We examined 43 patients with glomerulonephritis having renal biopsy. Sixteen of the 43 patients were examined before and during the treatment with predonisolone. Chymase positive mast cells stained by immunohistochemistry and tubulointerstitial damage were evaluated in specimens obtained by renal biopsy. Using Doppler sonography, we measured resistive index for assessment of disturbance of renal circulation.

Results: The number of chymase positive mast cells was significantly correlated with the score for tubulointerstitial damage (Rs = 0.46, P = 0.002, n = 43) and the resistive index (R = 0.69, P < 0.001, n = 43). These three parameters and proteinuria were concomitantly decreased in the 16 patients treated with predonisolone. The decrease in the number of the chymase positive cells were significantly correlated with the decrease in resistive index (Rs = 0.69, P = 0.008, n = 16), but not statistically with the decrease in the score of damage within about 3-month-treatment.

Conclusions: These findings in patients with glomerulonephritis indicate that renal circulation relates to appearing of the chymase positive mast cells. Mast cell chymase correlated with the tubulointerstitial damage, but could not be proved as a cause of the tubulointerstitial fibrosis in this 3-month-observation.

W-PO20006

A STUDY OF RETROSPECTIVE ANALYSIS TO THE MORBILITY AND PATHOLOGICAL CHANGE OF 486 IGA NEPHROPATHY PATIENTS IN CHINA

JIANGHUA CHEN[1], XUELIN HE[1], HUIPIN WANG[1], QUN LI[1], DONGBO WU[1], JIONG TIAN[1]
The Kidney Disease Center, The First Affiliated Hospital of Medical College, Zhejiang University, Hangzhou, China[1]

Retrospectively analyzed the data of renal biopsy pathological report and general status of 486 hospital IgA nephropathy patients from July, 2001 to December, 2004 in our center. The percentage of IgA nephropathy was 39.67%. Sex: male was 249(51.23%), female was 237(48.77%). Age distribution : juvenal group was 43(8.85%), young adult group was 234(48.15%), middle age group was 150(30.86%), old age group was 59(12.14%). The average of the glomerulus in 486 renal biopsy patients was 18.78 ± 7.25, patients with sclerosing glomerulus was 274 case (56.38%), the average of sclerosing glomerulus was 1.98 ± 1.03. Patients with crescent was 105 case (21.60%), the average of crescent was 0.48

± 0.63. Patients with renal tubule atrophy was 308 cases (63.28%). According to Lee's classification patients with pathological change in level I was 63(21.60%), in level II was 73(15.02%), in level III was 129(26.54%), in level IV was 147(30.25%), others was 74(15.23%). Immune complex deposit: beside IgA, patients with other immune complex deposit in mesangial region was IgG 60 cases (12.36%), IgM 310 cases (63.75%), C3 367 cases (75.56%), C4 35 cases (7.13%), C1q 26 cases (5.26%), fibrinogen 289 cases (59.53%) in turns. It suggested that there was no significant difference between male and female patient ratio, the distributional difference of the patient age was large but the main was young and middle age patients, pathological change was various, renal tubule atrophy, sclerosing glomerulus and crescent could be seen, the major pathological levels were level III and IV, beside IgA, the most common immune complex deposit in mesangial region was C3 (75.56%), then IgM (63.75%) and fibrinogen (59.53%) in turns.

W-PO20007

ANGIOTENSIN II BLOCKADE AMELIORATES HYPERTENSIVE TUBULOINTERSTITIAL DISEASE: ITS ANTIFIBROTIC EFFECT VIA PAI-1 INHIBITION

JING SHAO[1], TOSHIO MIYATA[2], REIKO INAGI[2], TOSHIRO FUJITA[1], MASAOMI NANGAKU[1]
Division of Nephrology And Endocrinology, University of Tokyo School of Medicine, Japan[1], Institute of Medical Sciences, Tokai University School of Medicine, Japan[2]

Plasminogen activator inhibitor-1 (PAI-1) is a serine protease inhibitor that was first recognized as an inhibitor of intravascular fibrinolysis. It has recently been thought to play a role in deterioration of renal diseases, including glomerulosclerosis and tubulointerstitial fibrosis. In hypertension and end organ damage, the role of renin-angiotensin system (RAS) has long been studied. The RAS is now recognized to be linked to induction of PAI-1. This study focused on the pathogenesis of the interaction between PAI-1 and RAS.
Hypertensive kidney disease was induced by continuous infusion of 435 ng/kg/min of angiotensin II (Ang II) via subcutaneous osmotic mini-pumps in rats for two weeks. These rats developed hypertension documented by a 2-fold elevation of systolic blood pressure, proteinuria (63.6 ± 7.3 mg/24 h vs. 10.6 ± 1.1 mg/24 h, vehicle rats, $P < 0.05$), an alternation in renal function, and tubulointerstitial damage with de novo expression of alpha-smooth muscle actin (alpha-SMA). We also found an up-regulation of PAI-1 expression in renal tubulointerstitium.
To investigate whether the action of angiotensin II was mediated by its receptor, we fed the rats with 10 mg/kg body weight of olmesartan, an angiotensin II type 1 receptor blocker (ARB). Administration of this medicine effectively decreased the blood pressure (126 ± 5.6 mmHg vs. 198.9 ± 21 mmHg, Ang II-infused rats, $P < 0.05$) and ameliorated the manifestations of the disease. The treatment of ARB in Ang II-infused rats resulted in a decrease of PAI-1 expression in tubulointerstitium. Histological and immunological analysis also demonstrated the improvement of the disease.
The results suggest that PAI-1 plays a pivotal role in hypertensive nephropathy. ARB may have another regulatory action on fibrotic/fibrinolytic system mediated by PAI-1. This study provides evidence that the blockade of the regulation of PAI-1 is a novel target for the therapy of hypertensive tubulointerstitial disease.

W-PO20008

AN EXTREMELY HIGH DOSE OF LOSARTAN (L) PRESERVES FILTRATION BARRIER AND INTRARENAL DISTRIBUTION PATTERN OF ANGIOTENSIN II (AII) AND AT1 RECEPTOR (AT1R) IN THE REMNANT KIDNEY

LUCIANA FARIA DE CARVALHO[1], FUJIHARA[1], CK[1], MALHEIROS[1], DMAC[1], MATTAR[1]' AL[1], FERNANDES[1], CH[1], ZATZ[1]
Renal Division, Dep. Clinical Medicine, University of São Paulo, SP, Brazil[1]

Angiotensin II is important for the development of progressive nephropathies (PN) and may affect the filtration barrier. AII blockade efficiently prevents PN, proteinuria and the associated inflammation. We showed previously that the protective effect of the AT1R antagonist, losartan (L), is strongly dose-dependent. Here we investigated, in the 5/6 nephrectomy model (NX), whether a) proteinuria associates with loss of essential slit-membrane proteins, such as ZO1 and nephrin; b) barrier constituents are restored by late-onset L treatment; c) the possible renoprotective effect of L depends on its antihypertensive action. Adult male Munich-Wistar rats underwent Nx or Sham operation, remaining untreated for 30 days. Tail-cuff pressure (TCP, mm Hg), urinary albumin excretion (UalbV, mg/day), the extent of macrophage (MΦ) infiltration (cels/mm^2), the interstitial-to-tubular AT1R expression ratio (AT1R I/T), the interstitial (Int AII) and vascular (Vasc AII) AII expression, the percent glomerular area occupied by ZO-1 (% ZO-1) and Nephrin (% Neph) were determined at this time (NX30). The remaining rats were distributed among 3 additional groups: Nx60 (n = 13), receiving vehicle; L500 (n = 14), receiving L, 500 mg/L in drinking water; and HDZ + HCTZ (n = 14), receiving hydralazine, 24 mg/L, and hydrochlorothiazide, 6 mg/L, in drinking water. Measurements were repeated 60 days after Nx. Results in the Table.
Renal injury associated with hypertension, intense MΦ infiltration, massive proteinuria, interstitial shift of AT1R, intense interstitial AII expression and loss of barrier proteins. L500 reversed changes in TCP, UalbV and Nephrin expression, and returned M F, ZO-1, AT1R and Int AII expression to pretreatment values. These effects cannot be attributed to lowering of TCP, which also occurred in the HDZ + HCTZ group, and may reflect mostly the anti-inflammatory and antiproteinuric effects of L, as well as its favorable effect on slit membrane components.

	TCP	UalbV	MΦ	AT1R I/T	Vasc All	Int All	% ZO1	% Neph
Sham	166 ± 5	2 ± 0	64 ± 9	0.3 ± 0.1	5.7 ± 1.5	1.4 ± 0.4	73 ± 3	72 ± 2
NX 30	210 ± 6[a]	66 ± 6[a]	168 ± 23[a]	0.6 ± 0.1	2.3 ± 0.6[a]	3.7 ± 0.7	60 ± 2[a]	58 ± 1[a]
NX 60	227 ± 5[a]	70 ± 7[a]	254 ± 20[ab]	1.5 ± 0.2[ab]	0.1 ± 0.1[ab]	7.2 ± 1.6[a]	57 ± 1[a]	59 ± 3[a]
L500	179 ± 7[abc]	26 ± 5[bc]	138 ± 13[c]	0.7 ± 0.1[c]	2.8 ± 0.8[ac]	5.4 ± 1.2[a]	62 ± 1[a]	70 ± 2[bc]
HDZ + HCTZ	163 ± 5[bc]	59 ± 10[ad]	207 ± 18[a]	1.1 ± 0.2[a]	0.4 ± 0.2[ad]	5.7 ± 1.1[a]	52 ± 2[ad]	61 ± 4[a]

(Mean ± SE, [a] $p < 0.05$ vs Sham; [b] $p < 0.05$ vs Nx 30; [c] $p < 0.05$ vs Nx60; [d] p < vs NxL500)

W-PO20009

PROGRESSION OF CHRONIC RENAL FAILURE IN IGA NEPHROPATHY: ROLE THE ANGIOTENSIN-CONVERTING ENZYME/ACE) GENE POLYMORPHISM

LUIS CARRERAS[1], FRANCESC MORESO[1], PEDRO ALIA[2], RAFAEL POVEDA[1], VERONICA DUARTE[1], JOSEP M. GRINYO[1]
Dpt. Nephrology. Hospital Universitario de Bellvitge. Barcelona. Spain[1]; Dpt. Biochemistry. H.U. Bellvitge[2]

We analyze the impact of ACE I/D polymorphism in a cohort of 112 patients diagnosed of Ig A nephropathy by renal biopsy. Cortical interstitial volume fraction was evaluated by means of a point-counting method. Genomic DNA was extracted from peripheral white blood cells and the detection of the insertion (I) and deletion (D) alleles of the ACE gene was done by PCR. We have used 113 control samples from healthy volunteers and kidney donors to study the distribution of ACE genotype. To evaluate the progression of chronic renal failure the slope of the 1/creatinine vs. time line was employed. Renal survival defined as dialysis free patients at the last follow up was calculated by the Kaplan Meier method.
There were 112 patients with a mean age of 42 ± 16 years, 88 males and 34 females. Mean follow up was 5.0 ± 5.4 years. ACE genotype distribution was not different between patients and controls (DD = 38%, ID = 48% and II = 16% vs. DD = 30%, ID = 55% and II = 15%). Clinical variables of the different genotypes are shown.

Variable	DD	ID	II	p
Gender (male/female)	30/12	41/12	12/4	ns[a]
Age	42 ± 15	42 ± 16	41 ± 10	ns[b]
Creatinine (umol/L)	153 ± 153	187 ± 187	127 ± 42	ns[b]
Proteinuria (g/day)	2.6 ± 3.1	1.7 ± 1.8	2.8 ± 2.6	ns[b]
Vvint/cortex (%)	6.8 ± 5.9	4.5 ± 2.8	4.6 ± 3.9	0.083[b]
Slope 1/Cr ($\times 10^{-5}$)	−3.4 ± 9.8	−2.0 ± 6.9	+0.3 ± 13.6	ns[b]
Renal survival (%)	59	88	92	0.031[c]

[a] Chi-squared, [b] ANOVA, [c] Log rank test.

To confirm the association between ACE polymorphism and renal survival we grouped patients as DD or non DD. Univariate (relative risk: 4.2, 95% confidence interval: 1.3–13.3, p = 0.016) and multivariate Cox regression analysis adjusting for age, proteinuria and creatinine (relative risk: 10.0, 95% confidence interval: 2.1–50.0; p = 0.004) confirmed that DD genotype is associated with a poorer outcome.
DD genotype is an independent predictor of end stage renal disease in IgA nephropathy.

W-PO20010

RENOPROTECTIVE EFFECT OF THE COMBINATION OF CLOPIDOGREL WITH IRBESARTAN

SUOZHU SHI[1], YUE GU[1], JIANJUN LI[1], XIANGMEI CHEN[1], ZHONG YIN[1], YANG LU[1], QUAN HONG[1]
General Hospita of PLA[1]

Background: platelet and angiotensin II (Ang II) participate in the pathogenesis of chronic nephropathy, including glomerular ECM accumulation, inflammatory reaction and disturbance of blood coagulation, through different ways. Inhibiting platelet activation or blocking Ang II delays the progression of renal disorder to different extents. Unfortunately, little is known about the combination effect of inhibiting both platelet activation and Ang II. The present study was to observe the renoprotective effect of the combination therapy of clopidogrel and irbesartan on 5/6 nephrectomized rats.
Methods: Wistar rats were subjected to 5/6 nephrectomy and were randomly assigned to 4 groups: untreated group, clopidogrel group (20 mg/kg/d), irbesartan group (20 mg/kg/d), and clopidogrel plus irbesartan group. Sham-operated rats served as controls. Rats were treated for 12 weeks. Twenty-four-hour urinary protein excretion (UP) and serum creatinine (SCr) were measured. Plasma GMP-140 was assessed by ELISA. Kidney histological changes were evaluated at the end of the study. Fibrinogen (Fib), fibroblast growth factor-2 (FGF-2), ICAM-1, PAI-1 and TIMP-1 protein and mRNA levels were examined.
Results: Compared with untreated group, clopidogrel or irbesartan reduced UP and SCr, decreased glomerular ECM accumulation and expressions of FGF-2, ICAM-1, PAI-1, and TIMP-1. Irbesartan was more effective than clopidogrel ($P < 0.05$). Clopidogrel significantly reduced GMP-140 and Fib deposition ($P < 0.05$). Irbesartan reduced GMP-140 and Fib in a far less pronounced way. Compared with clopidogrel or irbesartan, the combination therapy was more effective in reducing UP, SCr, glomerular ECM accumulation, Fib deposition and expressions of FGF-2, ICAM-1, PAI-1, and TIMP-1 ($P < 0.05$), while the combination therapy or clopidogrel showed no difference in plasma GMP-140 level.
Conclusion: Combination of clopidogrel with irbesartan was more effective in inhibiting renal glomerular ECM accumulation and inflammatory reaction than clopidogrel or irbesartan, indicating a synergetic renoprotective effect of clopidogrel and irbesartan.

W-PO20011

RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM AT EXPERIMENTAL RENAL HYPERTENSION

V.N. LUCHANINOVA[1], E.G. AGAPOV[1]
Vladivostok State Medical University, Russia[1]

Purpose: To study renin-angiotensin-aldosterone status at stages of development nephrosclerosis at experimental renal hypertensia.
Materials and Methods: Studied activity renin and aldosterone at white nonlinear rats (7 groups of animals on 7 in everyone). Researches carried out prior to the beginning, through 1; 3; 8 day, 2; 4; 8 and 24 weeks from the beginning of experiment.
Results: At the end of the first week at rats of researched group authentic increase of a level of activity renin in plasma (up to 8.73 ± 1.02 ng/ml/h) is marked in comparison with rats of control group (2.14 ± 0.34 ng/ml/h) ($p < 0.0001$). Simultaneously all animals had almost triple increase in activity aldosterone in plasma of blood (up to 296, 0.3 ± 23.9 ng/ml/h) in comparison with control parameters (103.5 ± 9.4 ng/ml/h) ($p < 0.0001$). At hypertensive rats within first four weeks stably high degree hyperreninemia and aldosteronemia ($p < 0.001$) was marked. In 8 weeks there was a decrease of activity renin up to 5.92 ± 0.68 ng/ml/h ($p < 0.001$) and growth of activity aldosterone (349.49 ± 26.9 ng/ml/h) ($p < 0.0001$). In 6 months from the beginning of experiment the level reninemia has decreased (3.11 ± 0.26 ng/ml/h), but did not reach control values and was authentic above them, that is a determinative in development of complications AH and remodeling of bodies – targets. Activity aldosterone in 6 months of experiment has reached the maximum and exceeded normative parameters in 4.7 times (489.84 ± 37.2 ng/ml/h) ($p < 0.0001$). On a measure of progressing of an arterial hypertensia dissociatia levels aldosterone and renin increased on a background of sclerous and dystrophic changes parenchyma kidneys and vessels.
Conclusion: Thus, basic pathogenetic mechanisms of development AH are similar to those in clinic and are based on changes of the contents in of blood renin – angiotensin and aldosterone.

W-PO20012

GENETIC MARKERS FOR PROGRESSION OF STEROID-RESISTANT NEPHROTIC SYNDROME IN CHILDHOOD

LARISA PRIKHODINA[1], ELENA ZAKLYAZMINSKAYA[2], NATALIA POLTAVETS[2], MAYA IGNATOVA[1], ALEXANDER POLAYKOV[2]
Pediatric Nephrology, Institute of Pediatrics & Children Surgery, Russian Federation[1], DNA laboratory, Research Center for Medical Genetics, Russia[2]

Steroid-resistant nephrotic syndrome (SRNS) is characterized by a high incidence of progression to chronic renal failure (CRF). We investigated whether gene polymorphisms of the rennin-angiotensin system (RAS) are associated with progression of SRNS in 22 Russian children (13M/9F; age 14.95 ± 0.51 years). Patients were genotyped for the angiotensin converting enzyme (ACE) I/D, the angiotensinogen (AGT) gene M235T and the type1 (A1166C) angiotensin II receptor (AT_1R) gene polymorphisms using polymerase chain reaction.
Children were divided into 2 groups: 1st, 11 pts with CRF (median GFR 46.07 ± 0.18 ml/min/1.73 m^2), including membranoproliferative GN (MbPGN) (n = 5); focal segmental glomerulosclerosis (FSGS) (n = 4), mesangial proliferative GN (MPGN) (n = 2); 2nd, 11 pts with normal renal function (median GFR 117.66 ± 7.07 ml/min/1.73 m^2) who has had MPGN (n = 6), FSGS (n = 2), membranous nephropathy (n = 2) and MbPGN (n = 1). There was no difference in the duration of SRNS in children of both groups (4.55 ± 2.13 vs. 5.25 ± 1.39 yrs).
Patients with CRF in comparison to the children with normal GFR have more frequent the following genotypes: ACE D/D (27.3% vs. 9.1%), AGT M235T M/T (72.7% vs. 45.4%) and AT_1R A1166C A/A (63.6% vs. 36.41%). In children with CRF in comparison to non progressors patients we also found increased value of proteinuria (6.9 ± 0.7 g/l vs. 3.1 ± 0.9 g/l), BP (140.5 ± 89.5 vs. 123.0/77.0) and frequency of left ventricular hypertrophy (81.8% vs. 36.4%).
These date demonstrate that progression of SRNS to CRF influenced by genetic conditions associated with high expression of molecules of the RAS, including ACE D/D, AGT M235T M/T and AT_1R A1166C A/A polymorphisms.

W-PO20013

ADENOSINE A1 RECEPTOR ANTAGONISM REDUCE PROTEINURIA BY INTERACTION WITH RENIN ANGIOTENSIN SYSTEM IN DAHL SALT-SENSITIVE RATS

CHUN-HUA JIN[1], TAKEFUMI MORI[1], MASAYUKI KANAZAWA[2], TETSUYA OOTAKA[3], HIROSHI SATO[1], MASAHIRO KOHZUKI[1], SADAYOSHI ITO[1]
Division of Nephrology, Endocrinology, and Vascular Medicine, Tohoku University Graduate School of Medicine[1], Department of Internal Medicine and Rehabilitation Science, Tohoku University Graduate School of Medicine[2], School of Health Sciences, Faculty of Medicine, Tohoku University[3]

Previous reports have shown that adenosine (Ado) interacts with renal renin-angiotensin system (RAS) and play a role in glomerular function. Present study was designed to investigate whether Ado A1 receptor antagonist FK838 (FK, 1 mg/kg, orally) could reduce proteinuria dependent of renin angiotensin system in Dahl salt sensitive (DahlS) rats.
6 weeks old male DahlS rats were assigned to six groups and caged under diets and treatments as below; A) normal salt diet (0.3% NaCl) group, B) 8% salt diet group, C) 8% salt diet + FK group, D) 8% salt diet + enarapril (ENA, 2 mg/kg, subcutaneusly) + FK group, E) 8% salt diet + enarapril (ENA, 2 mg/kg, subcutaneusly) + Furosemide (Fur, 10 mg/kg, orally) group, F) 8% salt diet + ENA + FK + Fur group. Urine was collected in a metabolic cage for every two weeks up to 6 weeks to measure urinary electrolytes and protein excretion. Systolic blood pressure (SBP) was measured by tail cuff method for every 2 weeks. SBP of rats under 8% salt diets was increased compared to that of 0.3% salt diet. Small but significant reduction in SBP was seen only when FK was administered together with ENA or with ENA and Fur. FK alone failed to alter increase in SBP under 8% salt diets. Rats under 8% salt diets alone had increased urinary protein excretion while those with 0.3% salt had no significant increase. Reduction of proteinuria was observed in rats treated with FK alone and addition of ENA did not show further reduction. Administration of FK to rats with ENA and Fur significantly reduced proteinuria while those with ENA and Fur failed, indicating that FK can further reduce proteinuria also under volume depleted state.
We conclude that blockade of Ado A1 receptor could reduce proteinuria independent of reduction in blood pressure and would interact with renin-angiotensin system, which in turn could play a role in protecting from renal injury in DahlS rats.

W-PO20014

COMPLEMENT C5 CONTRIBUTES TO EXPERIMENTAL TUBULOINTERSTITIAL RENAL FIBROSIS

JÜRGEN FLOEGE[1], ANDRZEJ KONIECZNY[1], TAMMO OSTENDORF[1] EVA BÜCHER[1], SONG RONG[1], UTA KUNTER[1], CLAUDIA VAN ROEYEN[1], SONJA HILLEBRANDT[2] FRANK LAMMERT[2], FRANK EITNER[1], JÜRGEN FLOEGE[1]

Nephrology And Clinical Immunology, Aachen University Clinic, Germany[1]; Medicine I, University Hospital Bonn, Germany[2]

Renal fibrosis characterizes the common endpoint of most renal diseases leading to terminal renal failure. Consequently, such final common pathways are particularly attractive targets for therapeutic intervention. We previously identified C5 as the fibrosis risk-associated gene underlying the *Hfib* locus on chromosome 2 and confirmed the association between C5 and liver fibrosis.

In the present study we investigated the role of C5 in the development of tubulointerstitial renal fibrosis by inducing renal fibrosis in wildtype and C5–/– mice by unilateral ureteral ligation (UUO). Obstructed and contralateral non-obstructed kidneys were examined on days 5 and 10 after the operation.

Renal fibrosis was first assessed by Sirius-Red staining and computer-assisted morphometry. In the C5–/– mice, as compared to the fibrotic wildtype control group, the positively stained cortical area was significantly ($p < 0.05$) reduced on day 5 by 53% (13 % vs. 28 % area staining with Sirius-Red). Similarly, cortical type I collagen immunostaining was reduced by 52% (11.8 vs. 24.5) and cortical a-smooth-muscle actin, a marker of myofibroblasts, by 43% (10.8 vs. 18.8) on day 5 after disease induction. On day 10 after disease induction, fibrosis was extensive in both groups and none of the above differences persisted. Tubulointerstitial type IV-collagen protein expression did not differ at any time point. These results were corroborated by real-time RT-PCR analysis of cortical mRNA of type IV collagen (no changes on days 5 and 10) and fibronectin (significant reduction on both days in C5–/– mice vs. wildtype mice). Finally, the cortical mRNA expression of all four members of the Platelet Derived Growth Factor (PDGF) family, which have been previously demonstrated to be central mediators of renal disease, was significantly reduced in the C5–/– mice.

Introduction: In conclusion, our data identify C5 as a novel pro-fibrotic factor in renal disease and as a potential target of future therapeutic strategies.

W-PO20015

COMBINED EFFECT OF LOSARTAN AND PRAVASTATIN ON INTERSTITIAL INFLAMMATION AND FIBROSIS IN CHRONIC CYCLOSPORINE NEPHROPATHY

CAN LI[1], SUN WOO LIM[2], SHIN WOOK KANG[3], YU SUN KIM[3], DUK HEE KANG[4], JIN KIM[5], CHUL WOO YANG[2]

Internal Medicine, YanBian University Medical College, China[1], Internal Medicine, The Catholic University of Korea[2], Internal Medicine, Yonsei University College of Medicine, Korea[3], Internal Medicine, Ewha Women's University College of Medicine, Korea[4], Anatomy, The Catholic University of Korea, Korea[5]

Background: Statins and angiotensin II type I receptor blockers have synergistic effects on vascular smooth muscle cells proliferation and the progression of renal diseases. We evaluated whether combined treatment with losartan (LSRT) and pravastatin (PRVT) affords superior protection compared with their respective monotherapies in treating chronic cyclosporine (CsA)-induced nephropathy in rats.

Methods: Rats maintained on a low salt diet were given vehicle, CsA (15 mg/kg), CsA and LSRT (10 mg/kg), CsA and PRVT (5 mg/kg), or a combination of CsA, LSRT, and PRVT for 28 days. Basic parameters (renal function, systolic blood pressure, serum high sensitivity C-reactive protein [hs-CRP], and lipid profiles), histopathology (arteriolopathy, tubulointerstitial fibrosis, and inflammatory cell infiltration), and inflammatory and fibrotic factors (intrarenal CRP, angiotensin II, osteopontin, and transforming growth factor [TGF]-b1) were studied.

Results: LSRT or PRVT treatment significantly attenuated the histopathologic changes induced by CsA, and combined treatment with LSRT and PRVT further decreased these parameters compared with giving each drug alone. Increased levels of angiotensin II, intrarenal CRP, osteopontin, and TGF-b1 in CsA-treated rat kidney were reduced by treatment with either LSRT or PRVT, and were further decreased by the combination of the two drugs. There were no significant differences in systolic blood pressure or serum lipid parameters between groups.

Conclusions: Combined treatment with LSRT and PRVT provided synergistic effects in attenuating inflammatory and fibrotic processes in a rat model of chronic CsA-induced nephropathy, and this effect was independent of their hypolipidemic and hypotensive actions.

W-PO20016

ANGIOTENSIN-CONVERTING ENZYME (ACE) GENE POLYMORPHISMS AND LUPUS DISEASE SEVERITY: A PROMISING LINK

MALIK ANAS RABBANI[1], SAEED MOHAMMAD[2]

Section Nephrology, Department Of Medicine, Aga Khan University Hospital, Karachi, Pakistan[1], Biological And Biomedical Sciences, Aga Khan University Hospital, Karachi, Pakistan[2]

We hypothesized that genetic sequence variation of *ACE* may not be causative for SLE, but may be involved with disease progression. We carried out a retrospective, case-control study of two polymorphisms for putative associations with SLE and allied phenotypes in a sample population of 39 SLE patients and 79 matched healthy control subjects. We used Systemic Lupus Activity Measure score at diagnosis as an indicator of disease severity (SLAM score 20 indicating severe SLE and 10, mild disease). Genotyping for *ACE* I/D and 2350 G > A polymorphisms was done. Differences in the distributions of six genotypes were not statistically significant for either of *ACE* polymorphisms, as assessed by chi-squared analyses. Groups were in Hardy-Weinberg equilibrium for both markers as determined by D_A statistics. Frequency of 2350A allele increased from 17% in mild SLE to 28% in moderate disease to 32% in severe SLE. Haplotype analysis and LD statistics demonstrated that D and 2350A alleles were in strong linkage disequilibrium (LD) ($D = -0.23$, $D = 0.72$, $c^2 = 64.4$, $P < 0.001$). Extent of LD was more in severe SLE ($D = -0.52$, $c^2 = 5.04$, $P = 0.025$) than mild to moderate disease ($D = -0.26$, $c^2 = 1.42$, $P = 0.23$). DA haplotype was more frequent in severe SLE than mild to moderate disease (odds ratio = 1.43, 95% confidence interval = 0.38 5.35, $c^2 = 0.36$, 1 df, $P = 0.55$).

SNP	Geno-types/ Alleles	SLE patients (n = 39)	Controls (n = 79)	Association (χ^2(2df)/*P*)
ACE I/D	II/ID/DD I/D	14/14/11 0.54 ± 0.04/0.46 ± 0.04	27/38/14 0.58 ± 0.06/0.42 ± 0.06	2.26/0.32
D_A/χ^2		0.07/3.14	0.0008/<0.001	
ACE 2350 G>A	GG/GA/AA	18/20/1 0.72 ± 0.07/0.29 ± 0.07	38/35/6 0.70 ± 0.04/0.30 ± 0.04	1.41/0.49
D_A/χ^2		–0.059/3.06	–0.014/0.354	

ACE gene does not appear to be involved in the development of SLE as shown by lack of association of ACE I/D and G > A polymorphisms. Though frequency of 2350A allele was similar in both groups, its distribution was skewed towards severe. D and the 2350A alleles were in strong LD and the predominant transmission of DA haplotype in severe SLE indicated its segregation with severe SLE. These results support the involvement of *ACE* polymorphisms with increasing disease severity of SLE.

W-PO20017

THE ROLE OF RENIN ANGIOTENSIN SYSTEM (RAS) IN THE ANTIPROTEINURIC AND BP RESPONSES OF TYPE 2 DIABETIC NEPHROPATHY

CM CHAN[1], YY SEOW[1], Z ZHOU[3], KS WONG[1]

Renal Medicine, Singapore General Hospital, Singapore[1], Clinical Research, Singapore General Hospital, Singapore[3]

Introduction: The Renin–Angiotensin System has an important pathophysiological role in patients with Type 2 diabetes and the ACE D allele is associated with microangiopathic complications. The DD genotype may be a significant risk factor in the progression of Diabetic Nephropathy. However, the Atg M235T has not been associated with microangiopathy. The ATR1 receptor gene A1166®C physiological significance is not determined. We examined the role of the three genes in the antiproteinuric and BP responses to RAS blockade.

Methods: In this retrospective study we evaluated 92 Type 2 Diabetic patients9renal impairment with proteinuria >1 g) in a government funded Renal Retardation Programme. All patients have renal impairment with significant proteinuria (>1 g per day). The ACE genotypes, Atg M235T and T174M and the A1166®C genotypes were examined in these patients. The antiproteinuric and BP responses were investigated at baseline and at 9 months.

Results: The frequencies of ACE II/DD/ID genotypes were 46%, 10% and 44%. The frequencies for AT1 A1166®C gene were 85% for AA and 15% for AC genotype. The frequencies for Atg M235T genotype (TT/MT/MM) were 6%, 28% and 67%. The Atg T174M genotypes (TT/MT/MM) were 2%, 20% and 78%.

The mean proteinuria (95% CI) at baseline and 9 months were 3.3(2.6 to 4.1) g/day and 2.6(2.0 to 3.3). The reduction in proteinuria at 9 months was significant with $P < 0.005$. The mean systolic BP (95% CI)/diastolic BP at baseline and at 9 months were 145(141 to 150)/83(81 to 85) and 137(133 to 140)/75(73 to 77). The reduction in systolic and diastolic BP at 9 months were significant with $P < 0.005$.

There was a trend in the antiproteinuric effect (<50% reduction, non-responder) in patients with ACE II genotype and AT1 AA genotype.

Conclusion: The RAS gene polymorphism maybe an important predictor of response to RAS blockade.

W-PO20018

THE EFFECT OF VALSARTAN ON SLOWING PROGRESSION OF RENAL FAILURE IN PATIENTS WITH ADVANCED RENAL FAIL

HIDEKI TAKIZAWA[1], IZUMI YAMAJI[1], TAMAKI TAKADA[1], YASUHIRO OOMOTO[1], HIRONORI MURAKAMI[1], SHIGEMICHI TANAKA[1]

Department of Nephrology and Cardiology, Teine Keijinkai Hospital, Japan[1]

Objective: Studies on the efficacy of angiotensin receptor blockade in preventing progression of renal failure have been focused on subjects whose serum creatinine is less than 3.0 mg/dl (265 μmol/l). However, this study was directed at evaluating the effect of angiotensin receptor blocker valsartan on slowing progression of renal failure in patients with a serum creatinine greater than 3.0 mg/dl.

Design and Methods: From our hospital database, we identified patients with renal failure ($n = 108$) not treated with valsartan and graphed their serum creatinine versus time to establish mean time-to-dialysis. Separately, 13 consecutive hypertensive patients with renal failure whose serum creatinine was between 3.0 and 5.9 mg/dl (mean 4.1 mg/dl) were treated with valsartan (40 to 80 mg once daily) in addition to conventional anti-hypertensive treatment for 12 months. We graphed their serum creatinine versus time, and used this to predict their time-to-dialysis based on the time at which their creatinine would be expected to achieve the aforementioned dialysis-requiring threshold creatinine. We compared the predicted time-to-dialysis in valsartan-treated patients to creatinine-matched patients in the non-valsartan database.

Results: In 12 of 13 patients of the valsartan group, the predicted time-to-dialysis was extended compared to non-valsartan database (mean 1336 vs. 197 days, $p < 0.05$).

Conclusion: Valsartan is effective in slowing progression of renal failure even in patients with advanced renal failure.

Pathogenesis Of Progression – Other Vasoactive Agents

W-PO20019

ADRENALECTOMY AMELIORATING REMNANT NEPHROPATHY IN THE RAT DEPENDS ON ALDOSTERONE LEVEL

LINGYUN LAI[1], YONG GU[1], JING CHEN[1], CHUANMING HAO[1], SHANYAN LIN[1]

Nephrology Research Institute and Division of Nephrology, Huashan Hospital, Fudan University, China[1]

The efficacy of ACEI in slowing the progression of renal disease has become widely accepted. More recently, aldosterone has also been implicated as a deleterious influence on the RAAS. This study, therefore, is tested the hypothesis that adrenalectomy ameliorates remnant nephropathy in the rat which depends on low aldosterone level.

Five groups of male Wistar rats weighing 200 grams were studied: SHAM rats, 5/6 nephrectomy rats, nephrectomy and adrenalectomized rats, bi-ectomized rats infused with exogenous aldosterone or dexamethasone by osmotic mini-pump at 40 μg/kg per d and 12 μg/kg per d, respectively. They were allowed free access to saline and sacrificed at 8 weeks. 5/6 rats had marked proteinuria, hypertension, and glomerulosclerosis as well as larger adrenal glands and accompanied with a >4-fold elevation in plasma aldosterone compared to SHAM rats. Above symptoms and renal histopathology were improved much in bi-ectomised rats with significant lower aldosterone level (less than the half of SHAM rats) (urinary albumin mg/24 h: 19.7 ± 2.0 vs. 5/6 rats 31.7 ± 1.7 $P < 0.001$; SBP mmHg: 173.8 ± 4.3 vs. 5/6 rats 210.4 ± 4.1 $P < 0.001$; glomerulosclerosis scores: 38.2 ± 7.9 vs. 5/6 rats 92.3 ± 6.7 $P < 0.001$). However, if we constantly infused exogenous aldosterone to bi-ectomized rats, they manifested greater proteinuria (urinary albumin mg/24 h: 24.9 ± 1.4 $P < 0.001$), hypertension (SBP mmHg: 201.5 ± 4.5 $P < 0.001$) and glomerulosclerosis (scores: 88.1 ± 7.2 $P < 0.001$) compared to bi-ectomised rats. Indeed, all of these features of the experimental disease were similar in exogenous aldosterone rats and 5/6 nephrectomy rats. But replacing with dexamethasone didn't deteriorate injury. Otherwise, GFR and kidney/ body weight were not different in four experimental groups.

Our data suggest that aldosterone, not glucocorticoid, contributes to the progression of ablative nephropathy in the rat through mechanisms more than systolic blood pressure.

W-PO20020

THE EFFECT OF METABOLIC ACIDOSIS ON THE RATE OF DECLINE OF GLOMERULAR FILTRATION RATE IN PATIENTS WITH STAGE 4 CHRONIC KIDNEY DISEASE

IONE DE BRITO ASHURST[1], MIRA VARAGUNAM[2], MARTIN J RAFTERY[2], STAN FAN[2], MOHAMMED MAGDI YAQOOB[2]

Barts and The London NHS Trust[1], Royal London Hospital[2]

We conducted this observational study to investigate the effect of persistent metabolic acidosis on the rate of decline of renal failure patients with stage 4 CKD, with time averaged bicarbonate between 15–20 mmol/l over the previous 2 yrs. Ninety patients (37 F) with CKD and GFR of <30 ml/min with diabetic nephropathy ($n = 28$), primary glomerulonephritis ($n = 5$) and other causes ($n = 57$), with two years of dialysis free follow up, were studied prospectively for rate of progression of renal failure by serial measurements of estimated GFR using the Cockroft-Gault equation, blood pressure (BP), body weight (wt), haemoglobin (Hb) serum PTH, calcium (Ca), phosphate (PO4), alkaline phosphatase (ALP), albumin, C-reactive protein (CRP), urea and creatinine level every 2 months. Proteinuria/day was analysed yearly.

Group 1 comprised 35 patients with acidosis and group 2 comprised 55 patients with average bicarbonate >20 mmol/l. The two groups were comparable in age, gender and cause of renal failure. Baseline and serial BP, wt, Hb, PTH, Ca, PO4, ALP, Albumin, CRP and proteinuria were similar in both groups. However, the rate of decline of GFR was significantly higher in group 1 from a baseline GFR of 20.50 ± 1.09 to 13.92 ± 0.99 over two years ($p < 0.001$). In group 2 there was no significant decline in GFR ($19.79 + 0.93$ to $17.13 + 0.88$; p = ns). Univariate and multivariate Cox regression analysis confirmed acidosis as an independent predictor of rate of decline of GFR in all patients except in those with diabetic nephropathy.

We conclude that low grade acidosis in patients with stage 4 CKD and optimal BP is associated with more rapid progression of renal failure. In the absence of acidosis, the rate of decline of GFR of 1 ml/min/year may simply be age related and might therefore be regarded as remission.

W-PO20021

PROTECTIVE EFFECT OF RESVERATROL IN 5/6 NEPHRECTOMIZED RATS

VIKAS CHANDER[1], DR. KANWALJIT CHOPRA[1]

University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh-160014, India

Background: The renal protective effect of resveratrol, a polyphenolic phytoalexin, was investigated in the 5/6 nephrectomized rats.

Methods: Resveratrol (5, 10 mg/kg, p.o.) was administered for 12 weeks to 5/6 NX rats together with and without nitro L-arginine methyl ester (L-NAME) (10 mg/kg, i.p). We evaluated the effect of these agents on proteinuria, hypertension, renal function, glomerulosclerosis and urinary excretion of nitric oxide (NO) metabolites.

Results: 5/6 NX resulted in elevation in systolic blood pressure (SBP), reduced the urinary excretion of NO metabolites, increased urinary protein excretion and glomerulosclerosis. Treatment of animals with resveratrol significantly attenuated the increase in SBP, preserved the normal renal function, reduced the urinary protein excretion, increased the urinary excretion of NO metabolites and pre-

vented the glomerulosclerosis. Treatment of animals with L-NAME along with resveratrol prevented the protection observed with resveratrol.
Conclusion: These findings indicates that resveratrol exerts its protective effect in 5/6 NX rats through a nitric oxide pathway.

W-PO20022

PPARGAMMA LIGANDS-INDUCED SODIUM RETENSION IS ASSOCIATED WITH RENAL CYTOCHROMEP450 IN OVARIECTOMIZED OBESE RATS

KYOKO YOSHIOKA[1], SHU WAKINO[1], KOICHI HAYASHI[1], TAKESHI KANDA[1], KOICHIRO HOMMA[1], SATORU TATEMATSU[1], ICHIRO TAKAMATSU[1], KAZUHIRO HASEGAWA[1], NAOKI SUGANO[1], OSAMU ITOU[2], KEN OMATA[3]
Internal medicine, Department of Medicine, Keio University[1], Depatment of Internal Medicine and Rehabilitation Science, Tohoku University Graduate School of Medicine[2], Health Administration Center, Miyagi University of Education[3]

Background: Although a PPARg ligand, pioglitazone (PIO), is reported to induce edema especially in postmenopausal women, its mechanism has not been clarified. We examined the effects of PIO on renal sodium excretion in female Zucker obese rats.
Methods: Rats were randomly assigned to the following groups: sham operated (Sham); ovariectomized (OVX); sham given PIO (Sham + pIO); OVX given PIO (OVX + pIO). After eight weeks, body weight and blood pressure were measured. After blood and urine samples were collected, kidneys were harvested to evaluate the renal expressions of neuronal nitric oxide synthase (nNOS), endothelial NOS (eNOS), cyclooxygenase-1/-2 (COX-1/-2) and CYP4A which metabolite is a potent stimulator of renal sodium excretion. Urinary NOx excretions were evaluated using Griess reaction.
Result: Blood pressure was significantly reduced in the rats treated with PIO than in untreated rats ($p < 0.05$). PIO decreased fasting blood glucose levels in each groups, although PIO reduced serum insulin levels only in OVX group. Sodium excretion was decreased in OVX + pIO rats compared with OVX rats ($p < 0.05$). On the contrary, urinary NOx excretion in OVX rats was less than in Sham rats, and was increased with PIO ($p < 0.05$), whereas the expressions of nNOS, eNOS, COX-1 and -2 were not altered by PIO. In contrast, western blot analysis using kidney homogenates revealed that the expression of CYP4A was increased in OVX rats and downregulated in OVX + pIO rats compared with OVX rats ($p < 0.05$). In vitro analysis using renal tubular cell line revealed that PIO downregulated the expression of CYP4A in a dose dependent manner.
Conclusion: Ovariectomy decreases renal NO production, but upregulates renal CYP4A expression telelogically to compensate for renal sodium balance. In this setting, PIO downregulates CYP4A, thus leading to sodium retension. The lack of ovarian function may determinate the PPARg-mediated sodium homeostasis in obesity.

W-PO20023

RENOPROTECTIVE EFFECT OF THE COMBINATION OF EPLERENONE AND ACE INHIBITOR VIA INHIBITION OF NADPH OXIDASE

MARISTELA LIKA ONOZATO[1], AKIHIRO TOJO[1], NAOHIKO KOBAYASHI[2], ATSUO GOTO[1], HIROAKI MATSUOKA[2], TOSHIRO FUJITA[1]
Division of Nephrology and Endocrinology, University of Tokyo, Tokyo, Japan[1], Division of Hypertension and Cardiorenal Disease, Dokkyo University School of Medicine, Tochigi, Japan[2]

Selective blockade of aldosterone is beneficial independent of renin-angiotensin blockade. We examined the effects of the aldosterone antagonist eplerenone on renal oxidative stress caused by NADPH oxidase in the Dahl salt-sensitive rats with heart failure (DSHF) and determined whether aldosterone antagonism would enhance the effectiveness of angiotensin converting enzyme (ACE) inhibition. DSHF rat were fed with 8% NaCl diet from 6 to 18 week-old. Eleven week-old DSHF rats received either vehicle, or eplerenone (DSHF + Eplerenone 30 mg/kg/day), or tandolapril (DSHF + ACEI 0.3 mg/kg/day) or a combination of both drugs for seven weeks. Renal NADPH oxidase, p47phox, superoxide production and renal damage were evaluated and scored 0 to 3 in each glomerulus and compared with the control Dahl salt-resistant rat fed with 8% NaCl diet. Blood pressure was increased in the DSHF rats (245 ± 5 vs. 132 ± 3 mmHg) and was reduced by ACE inhibitor (180 ± 4) and combined therapy (177 ± 5) but not by eplerenone alone (243 ± 5). In the kidney of DSHF rat, glomerular NADPH oxidase expression was increased (DSHF 0.77 ± 0.09 vs. 0.35 ± 0.07, $p < 0.005$) and promoted malondialdehyde deposition that showed a positive correlation with glomerulosclerosis ($R = 0.77$, DSHF 1.65 ± 0.17 vs. Control 0.26 ± 0.02, $p < 0.0001$). Eplerenone or ACE inhibitor reduced malondialdehyde and improved glomerulosclerosis (DSHF + Eplerenone 0.37 ± 0.08, $p < 0.0001$; DSHF + ACEI 0.94 ± 0.18, $p < 0.001$ vs. DSHF). Combination of eplerenone with ACE inhibitor further reduced NADPH oxidase (0.27 ± 0.03, $p < 0.0005$ vs. DSHF) and malondialdehyde to the control level and improved glomerulosclerosis (0.17 ± 0.02, $p < 0.0001$ vs. DSHF). The enhanced TGF-β and fibronectin in the DSHF rats were normalized with combined therapy. In conclusion, the aldosterone antagonist eplerenone combined with an ACE inhibitor reduced hypertensive glomerulosclerosis to the control level via inhibition of NADPH oxidase and reduction of blood pressure.

W-PO20024

ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH CORONARY HEART DISEASE AND CHRONIC GLOMERULONEPHRITIS IN CONTRAST MEDIA NEPHROTOXICITY

FATIMA U. DZGOEVA[1], ZAREMA U. DZGOEVA[1], ZAMIRA T.ASTAHOVA[2], KAZBEK D. SALBIEV[2], IRINA M. KUTYRINA[1]
Department of Nephrology, Moscow Medical Academy, Russian Federation[1]; Northen Osetian Medical Academy, Vladikavkaz Russian Federation[2]

Endothelial dysfunction is characterized by a shift of the actions of the endothelium toward reduced vasodilation and prothrombic properties. Mechanism that participate in the reduced vasodilatory responses in endothelial dysunction include reduced nitric oxide generation.
This study aimed to investigate whether NO synthesis in the acute nephrotoxic responses to constract media (CM) could be enhanced by simultaneous administration of calcium antagonist – verapamil. Morphologically confirmed model of acute renal failure (ARF) was induced by intravenous CM injection in rats.
The process of NO formation in the Kidney was monitored by the intensity of the electron (EPR) signal due to mononitrosyl iron complex (MNIC) formation with recorded in the tissues.
Renal blood volume, creatinine clearance, plasma, electrolytes (NA, CA, K), fractional excretion of sodium, plasma rennin activity, NO formation were determined before and 48 hours after CM injection.
There were three groups of animals: control – group I – were given the injection of isotonic saline, group II received CM (10 ml/kg), group III received both CM and calcium channel blocker – verapamil (1.5 mg/kg). The data 48 hours after injection indicate that verapamil prevented the rise in serum creatinne: group I – 101.1 ± 8.2 mmol/k (1.1 ± 0.12 mg/dl), group II – 259 ± 38 mmol/l (2.79 ± 0.7 mg/dl) and group III – 106.4 ± 6.8 mmol/l (1.24 ± 0.06 mg/dl), protected renal blood volume: 0.284 ± 0.5, 0.159 ± 0.2 and 0.269 ± 0.05 ml/g in group nI, II, III respectively; GFR – 0.8 ± 0.01, 0.59 ± 0.04 and 0.84 ± 0.08 ml/min/100 g in group I, II, III respectively; and also prevented the decrease of NO synthesis in kidney tissues: 5.3 ± 1.2, 3.2 ± 1 and 7.4 ± 2.8 ng/g in group I, II, III respectively. Our results suggest that NO inhibition aggravates ischemic form of AFR while stimulation of NO release exerts beneficial effect on renal hemodynamic parameters after ARF.
Protective effect of verapamil may be related to normalization of calcium – dependent NO-synthase activity.

W-PO20025

MOLECULAR MECHANISMS OF ENDOGENOUS NITRIC OXIDE SYNTHASE INHIBITOR ACCUMULATION IN CHRONIC RENAL FAILURE

KYOKO MATSUGUMA[1], SEIJI UEDA[1], SHO-ICHI YAMAGISHI[2], YOSUKE INAGAKI[2], RYO SHIBATA[1], YURIKO MATSUMOTO[1], SEIYA KATO[3], TSUTOMU IMAIZUMI[2], SEIYA OKUDA[1]
Nephrology, Kurume University School of Medicine, Japan[1], Internal Medicine III, Kurume University School of Medicine, Japan[2], Pathology, Kurume University School of Medicine, Japan[3]

Asymmetric dimetylarginine (ADMA) is an endogenous inhibitor of NO synthase (NOS). ADMA is generated by protein methyltranseferase (PRMT) and is mainly metabolized by dimethylarginine dimethylaminohydrolase (DDAH). ADMA is markedly increased in patients with chronic renal failure, and ADMA has been associated with atherosclerotic complications. However, the precise mechanism of ADMA accumulation in these patients is not fully understood. In this study, we investigated the relationship among renal function, blood pressure (BP), and methylated arginines in the rat remnant kidney model of progressive renal failure. We also explored renal DDAHs (DDAH-I and DDAH-II) expression and its activity. After Male Sprague-Dawley rats undergo baseline measurement of blood pressure and renal function, 5/6 subtotal nephrectomy (Nx) was performed. The plasma ADMA and SDMA, an inert isomer of ADMA, levels were significantly related to renal function. Renal clearance of ADMA was also elevated in the Nx group, whereas that of SDMA was reduced. Renal DDAHs expression was significantly decreased and PRMTs expression was incresed in Nx group. Moreover, the plasma ADMA level showed strong correlation with NOx excretion, BP, and heart weight. Our results suggest that decreased DDAH expression and increased PRMTs expression may augment ADMA accumulation and subsequent cause systemic hypertension and/or cardiac hypertrophy in rat model with progressive renal disease.

W-PO20026

HIGH LEVEL OF VCAM-I AND LOW LEVEL OF ADIPONECTIN MAY REPRESENT AN ARTERIOSCLEROTIC CHANGE IN HEMODIALYSIS PATIENTS

KOUSUKE OKUDA[1], OSAMU SAITO[1], TETSU AKIMOTO[1], YASUHIRO ANDO[1], SHIGEAKI MUTO[1], YASUSHI ASANO[2], EIJI KUSANO[1]
Division of Nephrology, Jichi Medical School[2], Director, Sashima Redcross Hospital, Japan[3]

Arteriosclerosis is known as one of the most risky factor for the prognosis of hemodialysis (HD) patients. In HD patients, however, the mechanism of high incidence of arteriosclerosis is not elucidated. In this study, we evaluated the relation of adhesion molecules CD146, VCAM-I and anti-arteriosclerotic factor adiponectin (ADN) to the ischemic heart disease (IHD), cerebral vascular accident (CVA), aortic calcification and arteriosclerosis obliterans (ASO) in HD patients. 165 patients (83 males and 82 females, age; 63.6 ± 10.6 years old, duration of HD; 149.4 ± 103.5 month) were examined. The levels of CD146, VCAM-1 and ADN were determined with ELISA. At first, we evaluated the relationship of these factors to clinical parameters such as age, gender, duration of HD, BMI, nPCR, kt/v and serum creatinine by step-wise ANOVA method. For the influence parameters we also performed Fischer analysis. In results, ADN only showed negative correlation to BMI, and low value in male patients (male; 15.8 ± 1.3, female; 24.4 ± 1.6 mg/ml, $p < 0.001$). In contrast, CD146 and VCAM-I have no relation to all clinical parameters, however, showed a correlation each other significantly (VCAM-I = 2224 + 1.872′CD146; $p < 0.001$). The level of ADN was decreased in ASO patients (ASO group; 7.8 ± 3.6 mg/ml vs. non ASO group; 22.1 ± 12.3 mg/ml, $p < 0.05$). The level of VCAM-I was increased in the patients complicated with aortic calcification (non calcification group; 2977.5 ± 183.5 vs. calcification group; 3445.9 ± 147.2, $p < 0.05$). In conclusion, high level of VCAM-I may be related to the aortic calcification, however, VCAM-I itself does not cause an ASO change. Low level of ADN may represent an important risk factor for ASO in HD patients.

Pathogenesis of Progression – Other Cytokines

W-PO20027

ASSOCIATION OF UTEROGLOBIN GENE G38A POLYMORPHISM WITH THE PROGRESSION OF IGA NEPHROPATHY

SOO-LIM CHUN[1], YON SU KIM[2], DONG-WAN CHAE[2], CURIE AHN[2], JIN SUK HAN[2], SUHNGGWON KIM[2], JUNG SANG LEE[2]
Seoul National University Boramae Hospital[1], Seoul National University College of Medicine[2]

Uteroglobin (UG) is a protein with immunomodulatory properties. We evaluated the influence of G38A polymorphism in UG gene exon 1 on progression of IgA nephropathy (IgAN). 240 Korean patients with IgAN who had a minimal follow-up of 4 years (mean ± S.D: 9.2 ± 4.1 years), were recruited. Patients were classified according to the slope of reciprocal serum creatinine into slow progressors (≥ -0.05 dL*mg^{-1}*year^{-1}, N = 170) and fast progressors (N = 70). 315 healthy subjects were analyzed as controls. The G38A polymorphism was determined by TaqMan 5′ nuclease assay. The genotype and allele frequencies were not different between patients and controls. Initial renal function, proteinuria, and hypertension did not differ significantly between IgAN patients with different genotypes. Patients with AA/GA genotypes showed a worse renal outcome in Kaplan-Meier analysis of renal survival ($P = 0.041$). The UG polymorphism remained an independent risk factor for progression in multivariate analysis (Cox regression model, HR for UG AA genotype: 2.19, 95% CI 1.11–4.35), including initial creatinine and hypertension. Our results suggest that UG gene G38A polymorphism is not associated with the development of IgAN, but is an important marker of progression in IgAN patients.

W-PO20028

EXPRESSION OF ALPHA-1 PROTEINASE INHIBITOR IN HUMAN RENAL TUBULAR EPITHELIAL CELL LINE HKC

GONGYAO TANG[1], YIPU CHEN[1], MEI ZHANG[1], JINGMING LOU[1]
Center of Nephrology, Institute of Clinical Medical Sciences, China-Japan Friendship Hospital, Beijing, 100029 P.R. China[1]

Objective: α1 proteinase inhibitor (API) is one of the major serine proteinase inhibitors in human plasma, synthesized primarily in the liver. However, this inhibitor has also been shown to be expressed in human blood monocytes, neutrophils, activated lymphocytes, human intestinal epithelial cells and the human alveolar epithelial cell line A549. Our present study aimed to determine if human renal tubular epithelial cell line (HKC) express α1 proteinase inhibitor.
Methods: (1) API protein in cultured HKC was detected by indirect immunofluorescence staining. (2) The mRNA expression of API was detected with reverse transcription-polymerase chain reaction (RT-PCR). (3) PCR product was analysed by DNA sequencing.
Results: Indirect immunofluorescence staining showed that HKC was API positive. RT-PCR showed a significant band (533 bp) of API mRNA from HKC. The PCR product was sequenced and the sequence is the same as API mRNA in gene bank.
Conclusion: human renal tubular epithelial cells may express α1 proteinase inhibitor.

W-PO20029

PRELIMINARY STUDIES ON THE POTENTIAL RELATIONSHIP BETWEEN TRPTASE-POSITIVE MAST CELLS AND RENAL INTERSTITIAL FIBROSIS WITH LUPUS NEPHRITIS

ZHAO TAN[1], CHEN YIPU[1], TIAN XUEFEI[1], DONG HONGRUI[1]
Center of Nephrology, China-Japan Friendship Hospital, Beijing, P.R: China[1]

Objective: To evaluate whether tryptase-positive mast cell (MCs) have a role in tubulointerstitial fibrosis of lupus nephritis (LN), and the relationship between the infiltration of MCs and expression of protease-activated receptor-2 (PAR-2), TGF-β1 and collagen type I(Col I) in LN.
Methods: Renal biopsy specimens from patients with LN (class III, IV, V, n = 10, respectively) were evaluated, minimal change diseases (n = 11) served as controls.
Results: MCs were mainly localized in the interstitium especially in the fibrosis region in three LN group patients compared with control ($p < 0.01$ respectively). The positive areas for PAR-2, TGF-β1 in the renal tubular epithelial cells (RTECs) were higher in three LN groups compared with control (PAR-2: class III and class IV $p < 0.01$; class V $p > 0.05$; TGF-β1: class III and class IV $p < 0.05$, class V $p > 0.05$). The positive areas for Col I in the renal interstitium was higher in three LN groups compared with control (class IV $p < 0.01$, class III and class V $p > 0.05$ respectively). There were significantly positive correlations between MCs and the positive areas of PAR-2, TGF-β1 as well as the positive areas of Col I (r = 0.504, 0.498, 0.632, $P < 0.001$ respectively). There was significantly positive correlation between the positive areas for PAR-2 and that for TGF-β1 in the RTECs (r = 0.748, $p < 0.01$). There was also significantly positive correlation between the PAR-2-positive cells and TGF-β1-positive cells in the interstitium (r = 0.728, $p < 0.01$). Immunofluorescence double-staining assay results showed that some tryptase-positive cells overlapped with PAR-2-positived cells or TGF-β1-positived cells in the renal interstitum.
Conclusion: The infiltration of MCs in the renal interstitum may have a role in tubulointerstitial fibrosis in LN by secreting trypatse → activation of PAR-2 → activation of TGF-β1.

W-PO20030

TUMOR NECROSIS FACTOR GENE G-308A POLYMORPHISM IN CHILDREN WITH NEPHROTIC SYNDROME

LASZLO KOVACS[1], LUDMILA PODRACKÁ[2], MICHAL HLADÍK[3], PAVOL GEIER[4]

Department of Pediatrics, Comenius University Medical School, Limbová 1, 83340 Bratislava, Slovakia[1], Department of Pediatrics, University Children s Hospital, Kosice, Slovakia[2], Department of Pediatrics, University Hospital, Ostrava, Czech Republic[3], Department of Pediatrics, University Hospital, Olomouc, Czech Republic[4]

Tumor necrosis factor (TNF) is a cytokine implicated in the severity of immune mediated diseases. Altered TNF production and/or receptor expression may play a role also in determining the clinical response to steroids in childhood nephrotic syndrome (NS). TNF gene G-308A polymorphism has been shown to be associated with enhanced cytokine production. Therefore, it is of interest to estimate the association of this polymorphism with steroid responsiveness/resistance of NS.

Methods: G-308A genotype of TNF gene was determined in 102 children with NS and in healthy controls using PCR and RFLP methodologies from a blood sample. Patients were retrospectively categorized into two groups: 78 had steroid sensitive NS (SSNS), while in 24 the NS was resistant to steroids (SRNS, 7 patients with minimal change NS and 17 with FSGS). Differences in the genotype distribution were calculated using chi-squared test.

Results: The prevalence of TNF A-308 allele in patients with NS did not differ significantly from that in controls (26.4% vs. 22.0%). However, the carrier frequency of the A-308 allele significantly differ between the SRNS and SSNS groups (12/24, 50.0% vs. 15/78, 19.2%, OR: 4.2, CI: 1.64–11.17, $p < 0.01$). There were no significant differences between these two groups as to gender distribution and age at onset of NS.

Conclusion: Steroid sensitivity is major determinant of prognosis in childhood NS. Elevated TNF gene expression and synthesis has been found in peripheral blood mononuclear cells (PBMC) from children with NS. Moreover, it has been suggested that increased in vitro TNF production from PBMC could be used to discriminate between children with SRNS and SSNS. Our results are in line with these findings by providing additional support to the hypothesis, that TNF might be involved in the pathological events occurring in non-inherited forms of childhood NS.

W-PO20031

TNF-ALFA, SOLUBLE INTERLEUKIN-2 RECEPTOR AND GLOMERULAR SCLEROSIS IN PRIMARY NEPHROTIC SYNDROME IN CHILDREN

ANNA EWA POGAN[1], KRYSTYNA SANCEWICZ-PACH[2], WITOLD MIEŻYŃSKI[3]

Department of Pediatric Nephrology, University Children's Hospital, Jagiellonian University Kraków, Poland[1]; Dpt. of Pediatric Nephrology Jagiellonian University Kraków, Poland[2], Dpt. of Pathology University Children's Hospital Kraków, Poland[3]

There are a great number of evidences, that primary immunological dysfunctions play a role in the ethiopathology of primary nephrotic syndrome (NS) in children.

The aim of the study was to evaluate the serum concentration of tumor necrosis factor alfa (TNF-alfa) and soluble interleukin 2 receptor (sIL-2R) in children with relapse of primary NS and to assess their prognostics for glomerular sclerosis.

Material and Methods: 98 children with relapse of primary NS, aged 1–16.5 years, 56 boys and 42 girls were enrolled into the study. Cytokines were determined in 141 relapses of NS. The kidney biopsy was performed in 51 patients, and repeated in 22. The control group consisted of 31 healthy children aged 0.6–16.5 years, 18 boys and 13 girls. The TNF-alfa and sIL-2R concentrations were measured by ELISA kits. Glomerular sclerosis was found in 19 children, mesangial proliferation in 4, minimal changes in 21, membranosa in 3, 4 cases stayed unclassified.

Results: TNF-alfa serum concentration increased in NS relapse. The risk for glomerular sclerosis was 3 times higher with increased over 2.35 pg/ml TNF-alfa concentrations. SIL-2R serum levels in a whole group did not differ from the control group. Lower sIL-2R concentrations below 529 pg/ml increased the risk (2.5 times) for glomerular sclerosis.

Conclusion: The risk of glomerular sclerosis increased with high TNF-alfa or low sIL 2R concentrations.

W-PO20032

CR002 (ANTI-PDGF-D ANTIBODY) INHIBITS MESANGIOPROLIFERATIVE GLOMERULONEPHRITIS (GN)

GLENNDA M. SMITHSON[1], GARY STARLING[1], WILLIAM LAROCHELLE[1], CLAUDIA VAN ROEYEN[2], TAMMO OSTENDORF[2], JÜRGEN FLOEGE[2]

CuraGen Corporation[1], University of Aachen[2]

Platelet derived growth factor-D (PDGF-D) is the most recently identified member of the PDGF family of growth factors. It is up regulated in glomeruli in experimental GN and induces mesangial cell proliferation in culture. Proliferation and activation of these cells occurs in IgA-GN, lupus nephritis and diabetic nephropathy and may be one of the early steps that initiate a cascade of events that ultimately leads to kidney failure.

We have generated a human anti-PDGF-D monoclonal antibody (CR002) using XenoMouse technology (Abgenix, Inc.) with a Kd of approximately $1.8 \times 10–10$ M. CR002 blocks mesangial cell proliferation *in vitro* and *in vivo* in a non-progressive rat anti-Thy 1.1 model of GN. In this acute model, there is a significant reduction in proliferating smooth actin positive mesangial cells, i.e. myofibroblast-like cells that are thought to be responsible for excessive extracellular matrix (ECM) deposition, and a reduction in glomerular ED-1+ monocytes and macrophages, as compared to controls. In a chronic (8 week) anti-Thy 1.1 model, induced by uni-nephrectomy and Thy 1.1 antibody in rats, treatment with CR002 on days 3, 10 and 17 inhibited focal segmental glomerular sclerosis, tubulointerstitial fibronectin accumulation and tubulointerstitial fibrosis at day 56 after disease induction. We, therefore, have evidence that CR002 blunts the progression of renal disease when given in the acute mesangioproliferative phase of glomerulonephritis.

Together, these studies suggest that PDGF-D plays an important role both in the initiation and progression of mesangio-proliferative kidney disease and that CR002 may provide an important novel treatment option in glomerular diseases characterized by mesangioproliferative changes.

W-PO20033

EFFECT OF FLUVASTATIN ON COLLAGEN TYPE IV AND MOLECULAR MECHANISM IN RATS OF GLOMERULAR SCLEROSIS

JIA LIU[1], WANG XIAOYUN[1], SUNBIN[1], XING CHANGYIN[1], PONG TAO[1], YE JIAN[1], GUO MEI[1]

Nanjing Medical University[1]

Objective: To investigate the effect of hydroxyl methylglulary coenzyme A reductase inhibitor (HCRI) fluvastatin on transforming growth factor-β_1 (TFG-β_1), plasminogen activator inhibitolr-1 (PAI-1) and collagen type IV in rats of glomerular sclerosis.

Methods: Five sixths nephrectomized rats were randomly divided into 4 groups: control group; treated with fluvastatin group (2 mg/kg·d); treated with benazepril group (6 mg/kg·d) and pesudonephreotomized group.Urinary volume, proteinuria, creatinine clearance rate (Ccr), serum creatinine, cholesterol and triglyceride were reviewed after 10 weeks of treatment. Renal tisstue were stained by HE and PAS and glomerular sclerosis index (GSI) were caculated while the expression of TGF-β_1, PAI-1 and collagen type IV protein were measured by the methods of western blotting and immunohistological staining respectively.

Results: proteinuria and the expression of PAI-1, TGF-β_1 collagen type IV, GSI in rats of fluvastatin and benazepril groups were significantly lower than the control ($P < 0.01$). Compared to control group, there was a significant increase in Ccr in groups treated with fluvastatin and benazepril ($P < 0.01$).

Conclusion: Fluvastatin not only reduces proteinuria, improves renal function, but also modulates glomerular sclerosis by inhibiting activity of TGF-β_1, PAI-1 and decreasing accumulation of collagen type IV. The mechanism of renal protective effect is independent of a reduction of circulating cholesterol.

W-PO20034

PROGNOSTIC FACTORS IN NON IGA MESANGIAL PROLIFERATIVE GLOMERULONEPHRITIS (MPGN)

PANAGIOTIS GIAMALIS[1], EFSTATHIOS ALEXOPOULOS[1], EKATERINI PAPAGIANNI[1], AFRODITE PANTZAKI[2], DIMITRIOS MEMMOS[1]

Department Of Nephrology, Hippokration Hospital, Thessaloniki[1], Department Of Pathology, Hippokration Hospital, Thessaloniki[2]

The aim of the study was to assess clinical, histological and immunohistochemical findings, at the time of renal biopsy, as predictors of outcome in patients (pts) with idiopathic MPGN. 31 MPGN pts, with mean age 43 years (range 22–62), were followed for 75 ± 50 months. Mean serum creatinine levels (SCr) on biopsy were 1.2 ± 0.6 mg/dl and mean proteinuria 3.2 ± 3 g/day. Renal biopsy specimens from all pts, were studied using monoclonal antibodies against C5b-9, monocytes/macrophages, proliferating cell nuclear antigen (PCNA), alpha smooth muscle actin, TGF-β1 and PDGF-BB. Mesangial cell proliferation and matrix expansion, interstitial inflammatory infiltration and fibrosis, were also evaluated. During follow up, 8 patients (25.8%) developed chronic renal failure (CRF), with SCr >1.5 mg/dl or GFR <60 ml/min/1.73 m^2. Pts progressing to CRF had higher levels of proteinuria ($p = 0.01$) and SCr ($p = 0.02$) on biopsy, as well as more severe interstitial fibrosis ($p = 0.03$) and inflammatory infiltration ($p = 0.03$). The number of interstitial PCNA(+) cells ($p = 0.01$) and PDGF-BB(+) cells ($p = 0.02$) and the interstitial expression of TGF-β1 ($p = 0.02$), were also more prominent in pts who developed CRF. The presence of IgM mesangial deposits and the degree of mesangial matrix accumulation did not correlate with CRF development. Interestingly, only 1/14 pts (7%) with mild mesangial cell proliferation developed CRF, in contrast to 7/17 pts (41%) with moderate or severe mesangial cell proliferation, although this difference was not statistically significant. In conclusion, clinical and histological variables may identify high-risk pts, for progression to CRF. Lower GFR levels on presentation were the only independent predictor of poor long-term prognosis. Acute proliferative phenomena [PCNA(+) cells, inflammatory infiltration] and chronic sclerotic lesions (TGF-β1 expression, interstitial fibrosis) in the tubulointerstitium, predict more accurately progression than glomerular lesions. Early interstitial cell proliferation [PCNA(+) cells] might serve as an index of disease severity and duration of treatment.

W-PO20035

INFLUENCE OF CYTOKINE GENE POLYMORPHISMS ON THE PROGRESSION OF PRIMARY GLOMERULONEPHRITIS

CHRISTOS BANTIS[1], PETER J HEERING[1], SENDOGAN AKER[1], NICOLA KUHR[1], BERND GRABENSEE[1], KATRIN IVENS[1]
Department of Nephrology, Heinrich-Heine University of Düsseldorf, Germany[1]

Introduction: Recently polymorphisms of cytokine genes have been associated with modified gene expression and increased cytokine production. We evaluated the influence of Interleukin-10 (IL-10) gene G-1082A, TGF-β1 gene $Arg^{25} \rightarrow$ pro, TNFα gene G-308A and IL-6 gene G-174C polymorphisms on the clinical course of primary glomerulonephritis.
Patients and Methods: We studied n = 251 patients with biopsy proven primary glomerulonephritis (IgA nephropathy: n = 127, focal segmental glomerulosclerosis: n = 71, membranous glomerulonephritis: n = 53) followed up for 6.2 ± 5.3 years. According to the slope of the curve of reciprocal serum creatinine against time (3 or <–0.1 dl*mg^{-1}*$year^{-1}$) group A (slow progressors, n = 162) and group B (fast progressors, n = 89) were defined. One hundred volunteers were analysed as controls. Genetic polymorphisms were determined by PCR amplification.
Results: The allele frequencies of the polymorphisms studied were similar in patients and control subjects (ns). Age, renal function, proteinuria and blood pressure did not differ significantly at the time of renal biopsy between patients with different genotypes (ns). IL-10 gene G-1082A polymorphism influenced the rate of progression, with the IL-10 low producer genotype (carriage of the A-allele) being associated with a worse prognosis: The low producer genotype was more frequent in group B (88.8%) than in group A (70.4%; $p < 0.001$). In the Kaplan Meier analysis of kidney survival patients with the IL-10 low producing genotype showed a significantly worse outcome (7.2 ± 0.7 years) compared to high producers (12.8 ± 2.3 years, mean ± SE, $p = 0.002$). The IL-10 genotype remained an independent risk factor for progression in multivariate analysis (Cox regression model: HR for the IL-10 low producing genotype: 1.46, 95.0% CI: 1.15 to 1.84, $p = 0.001$). TGF-β1, TNFα and IL-6 gene polymorphisms were not associated with any of the parameters studied.
Conclusion: Our results suggest that IL-10 gene G-1082A polymorphism is an important progression marker in patients with primary glomerulonephritis.

W-PO20036

ASSOCIATION OF CYTOKINE GENE POLYMORPHISMS WITH TYPE 2 DIABETIC NEPHROPATHY AND CHRONIC GLOMERULONEPHRITIS

NINA BABEL[1], CONSTANZE SCHOENENAMNN[2], MARKUS HAMMER[1], VOLODIMIR SKRYPNIKOV[1], HANS DIETER VOLK[3], PETRA REINKE[1]
Nephrology And Internal Intensive Care, Charite, Germany[1]*; Transfusion Medicine, Charite, Germany*[2]*; Medical Immunology, Charite, Germany*[3]

Background: There is mounting evidence suggesting an association between cytokine gene polymorphisms and increased risk of the progressive deterioration of renal function being the cause of end-stage renal disease (ESRD). With the premise that these polymorphisms may be possible genetic susceptibility factor for the progression of renal failure, we conducted a comparative study of cytokine gene polymorphisms in healthy subjects and haemodialysis patients.
Methods: The −1082IL-10, −308TNF-a, TGF-b1 (codon 10, 25), −174IL-6, +874IFN-g gene single nucleotide polymorphisms were studied by PCR-SSP in 120 healthy donors and 103 patients being on haemodialysis due to diabetic nephropathy or glomerulonephritis.
Results: Significant associations of the TGF-b1 (codon 10) TT and IL-10 (-1082) GG genotypes both with type 2 diabetic nephropathy and glomerulonephritis were found ($p < 0.001$ and $p < 0.01$, respectively). However, no significant differences in the TNF-a, IFN-g, IL-6 and TGF-b1 (codon 25) genotype distribution between healthy controls and patients with diabetic nephropathy- and glomerulonephritis-associated renal failure were detected.
Conclusions: Carriage of the TGF-b1 (codon 10) TT and IL-10(-1082) GG genotypes may be associated with increased susceptibility to diabetic nephropathy- and glomerulonephritis-associated ESRD. The fact that both types of primary renal disease (diabetic nephropathy and glomerulonephritis) were found to be associated with the same cytokine gene polymorphisms may suggest common pathogenesis of ESRD following these forms of initial renal impairment.

W-PO20037

LOSARTAN-INDUCED REDUCTION IN URINARY TGF-β1 CORRELATES WITH LONG-TERM RENOPROTECTION IN CHRONIC NON-DIABETIC NEPHROPATHY

HYEONG CHEON PARK[1], SHIN WOOK KANG[1], KYU HUN CHOI[1], SUNG KYU HA[1], HO YUNG LEE[1], DAE SUK HAN[1]
Internal Medicine, Yonsei University College of Medicine[1]

Urinary TGF-β1 reflects intra-renal TGF-β1 production that has a critical role in the pathogenesis of glomerular damage in chronic nephropathy. Aim of our study was to evaluate whether losartan, an angiotensin II receptor antagonist, induced reduction in urinary TGF-β1 might predict long-term renoprotection in nondiabetic chronic nephropathy. A total of 51 hypertensive patients with overt proteinuria and serum creatinine lower than 3 mg/dL were studied. The patients received losartan 100 mg daily and were followed prospectively for a mean period of 24 months. At baseline and at 6 months of treatment, clinical parameters and urinary TGF-β1 were determined. Patients were classified into responders (R, n = 39, more than 30% reduction) or nonresponders (NR, n = 12, less than 30% reduction) according to their antiproteinuric response at 6 month. Baseline proteinuria [geometric mean, (range)], mean arterial pressure, and creatinine clearance (CCr) values were similar between R and NR [R vs NR: 1599 (617–5619) vs 2572 (719–9382) mg/day, 106 ± 8 vs 103 ± 11 mmHg, 63 ± 25 vs 58 ± 19 ml/min, respectively]. Baseline urinary TGF-β1 excretion was significantly higher in NR (R vs NR: 28.0 ± 14.6 vs 49.6 ± 20.6 pg/mg creatinine, $p < 0.05$). The degree of blood pressure control, clinical and biochemical markers did not differ between R and NR. However, degree of urinary protein and TGF-β1 reduction (R vs NR: 65 vs 5.2% and 49 vs 21% at 6 months, respectively, $p < 0.05$) were significantly greater in R and degree of loss of CCr were significantly greater in NR (R vs NR: 18.8 ± 11.2 vs 9.9 ± 5.4 ml/min, $p = 0.05$). There was an inverse corrrelation between the percent change in urinary TGF-β1 level during the first 6 months and the percent change in CCr over the ensuing 2-year period ($r = -0.379$, $p = 0.019$). Our data suggest that losartan induced changes in urinary TGF-β1 level may predict the course of nondiabetic chronic nephropathy.

W-PO20038

AST-120 ABSORBS DIET-DERIVED AGES -A NOVEL ATHEROPROTECTIVE PROPERTY OF AST-120-

YURIKO MATSUMOTO[1], SEIJI UEDA[1], KEISUKE KOHNO[1], SHO-ICHI YAMAGISHI[2], MASAYOSHI TAKEUCHI[3], KYOKO MATSUGUMA[1], RYO SHIBATA[1], TSUTOMU IMAIZUMI[2], SEIYA OKUDA[1]

Department of Nephrology, Kurume University School of Medicine, Japan[1], *Department of Internal Medicine III, Kurume University School of Medicine, Japan*[2], *Department of Pathophysiological Science, Faculty of Pharmaceutical Sciences, Hokuriku University, Japan*[3]

Advanced glycation end products (AGEs), the senescent macroprotein derivatives which are formed in increased amounts in patients with diabetes and/or end-stage renal failure, may contribute to accelerated atherosclerosis. Recently, it has been demonstrated that orally absorbed AGEs play an important role in atherosclerosis and AGE-restricted dietary may be an effective inhibitor of atherosclerosis. In this study, we examined whether an oral absorbent (AST-120) could absorb the diet-derived AGEs. *In vitro*, about 15% of AGEs-modified BSA were absorbed by AST-120 for 2 h-incubation. Ten patients with chronic renal failure (age 59 ± 10 yrs, serum creatinine 4.3 ± 1.7 mg/dl) were enrolled in this study. All patients were kept on regular diet cure and medications throughout the study. The serum levels of AGEs were compared before and after three-month treatment with AST-120. Administration of AST-120 significantly reduced the serum levels of AGEs. Moreover, compared with the serum from these patients before AST-120 treatment, the serum after AST-120 treatment significantly reduced receptor for AGEs (RAGE) mRNA expression and atherosclerosis-related gene expression such as MCP-1 and VCAM by cultured human umbilical vein endothelial cells. Our present data indicates that AST-120 could reduce atherosclerosis-related gene expression by absorbing diet-derived AGEs, thus providing an atheroprotective property of AST-120.

Pathogenesis of Progression – Resident Cells

W-PO20039

DUAL EFFECT OF AN ALFA-ACTININ-4 GENE MUTATION ON PODOCYTE INJURY

TAIJI MATSUSAKA[1], AGNES B. FOGO[2], IEKUNI ICHIKAWA[2]

Vanderbilt and Tokai University[1], *Vanderbilt University Medical School*[2]

Alfa-actinin-4, an actin-crosslinking protein that contributes to dynamic integrity of actin filaments, is highly expressed in podocytes. K228E mutation in the alfa-actinin-4 gene has been associated with familial focal segmental glomerulosclerosis (FSGS), and the mutant protein has a higher affinity to F-actin than wild-type alfa-actinin-4. To investigate the in vivo consequence of the K228E mutation, we established four lines of transgenic (TG) mice (*Actn4*-TG) expressing similarly mutated mouse *Actn4* gene selectively in podocytes.

By 12 months of age, most *Atcn4*-TG developed FSGS with tubulointerstitial damage. The onset of the nephropathy varied among the lines, ranging from 2 to 12 months. Before the development of nephropathy, *Atcn4*-TG showed more intense actinin-4 immunoreactivities in podocytes than wild-type mice, which were downregulated after development of nephropathy.

To test whether an additional insult to podocytes accelerates the progression of FSGS, we induced podocyte injury in *Actn4*-TG mice. Thus, we mated *Actn4*-TG with NEP25, another TG line in which podocyte-restricted injury can be induced by administration of an immunotoxin, LMB2. 3 weeks after LMB2 (0.625 ng/g BW) administration, NEP25 carrying the mutant *Actn4* (n = 6) developed attenuated glomerular injuries than NEP25 without mutant *Actn4* (n = 11), with epithelial injury score of 0.12 ± 0.04 vs. 0.74 ± 0.20 (p < 0.01), on the scale of 0 to 4 for none to most severe injury. Expression of synaptopodin, a podocyte marker, was also better preserved in those with mutant Actn4. *Actn4*-TG without NEP25 transgene (n = 6) was still without abnormal renal histology at this age (10 weeks).

Collectively, although in a long run, the K228E mutation causes damage to podocytes, in a short run, it protects podocytes from insult by fortifying crosslinking of actin.

W-PO20040

INTEGRIN LINKED KINASE (ILK) EXPRESSION MAY BE USED AS AN INDICATOR OF PROGRESSION OF RENAL DISEASE IN EXPERIMENTAL AND HUMAN PROTEINURIC GLOMERULAR DISEASES

VICENTE DE PAULO TEIXEIRA[1], ANDREIA FABIANA VALE FRANCO[1], ROGERIO BARBOSA DE DEUS[1], NESTOR SCHOR[1], MARCELLO FABIANO DE FRANCO[1]

Universidade Federal de São Paulo[1]

Background: Glomerular diseases are the main cause of end stage renal disease and proteinuria is their hallmark. A common characteristic of these diseases is glomerular visceral epithelial cell or podocyte lesion. Recently, it was demonstrated that integrin-linked kinase (ILK) may function as a common downstream mediator in proteinuric diseases. ILK is a serine/threonine protein kinase that associates with integrins through its cytoplasmic subunit b 1, and is involved with several aspects of cell behavior including differentiation, repair and death.

Objective: The aim of this study was to investigate the ILK expression in human proteinuric glomerular diseases and an experimental model of progressive proteinuric glomerular disease in rats.

Methods: Renal biopsies from subjects with minimal change disease, membranous nephropathy and focal segmental glomerulosclerosis, and normal subjects were evaluated by immunohistochemistry for ILK expression. Male Wistar rats submitted to 5/6 nephrectomy (Nx 5/6) were sacrificed 2, 4, and 6 week after surgery. At each time period, serum creatinine, 24 h proteinuria, histopathologic changes and ILK expression were evaluated using Western blot and immunohistochemistry. ILK expression was also evaluated by immunofluorescence in several renal cell lines in culture.

Results: Renal biopsies of normal subjects presented ILK expression only in tubular region. On the other hand, there was strong ILK immunoexpression by podocytes from subjects with proteinuric renal disease proportional to the disease severity. Similarly, nephectomized rats developed strong ILK immunostaining in podocytes, in direct relation with the severity of the renal lesion. Furthermore, the Western blotting showed increased ILK expression in nephrectomized rats. All cell lines expressed ILK by immunofluorescence.

Conclusion: These data suggest that ILK might be involved in the pathogenetic mechanisms in experimental and human proteinuric renal disease. The ILK expression might be used as an indicator of progressive of renal disease.

W-PO20041

MITOCHONDRIAL DYSFUNCTION IN FOCAL SEGMENTAL GLOMERULOSCLEROSIS OF PUROMYCIN AMINONUCLEOSIDE NEPHROSIS

MASAHIRO HAGIWARA[1], KUNIHIRO YAMAGATA[2], YOH ARAKAWA[2], YOSHIO SHIMIZU[2], KEIGYOU YOH[2], KOUICHI HIRAYAMA[2], AKIO KOYAMA[2]

Department of Nephrology, University of Tsukuba, Japan[1]; *University of Tsukuba, Japan*[2]

Objective: We used puromycin aminonucleoside nephrosis (PAN), which is one of the focal segmental glomerular sclerosis (FSGS) models, to investigate the involvement of mitochondrial DNA (mtDNA) changes in the pathogenesis of FSGS.

Method: Two renal pathological phases were studied. A single injection of PA (nephrosis phase), and multiple injection of PA (FSGS phase) of PAN. We investigated the localization and amount of mtDNA encoded enzyme, cytochrome C oxydase subunit I (COX I) protein, and the copy number of 4.834 base-pair (bp) deleted mtDNA and wild–type mtDNA in glomeruli by quantitative real-time PCR in both phases of PAN. Furthermore we investigated the mRNA expression of several important mitochondrial biogenesis molecules, the mitochondrial transcription factor A (mtTFA) and the nuclear respiratory factor 1 (NRF-1) in PAN glomeruli.

Result: The protein expression level of COX I was identical to controls regardless of nephrosis phase. However, a reduction to 45% of controls was seen in FSGS phase glomeruli. Intra-glomerular 4.834-bp deleted mtDNA was 16 to 21 times higher than control rats in both phases, but the proportion of this mutation was less than 1% of controls. The copy number of wild-type mtDNA at nephrosis phase increased up to 241%, whereas, it decreased to approximately 34% at FSGS phase in glomeruli. The mRNA expression of both mtTFA and NRF-1 was up-regulated at nephrosis phase, but mtTFA was down-regulated at FSGS phase.

Conclusion: The lack of mtDNA copy number through the down-regulation of the mtTFA in glomeruli resulted in COX I protein reduction in glomeruli at FSGS phase of PAN. These results suggest that the pathogenesis of FSGS lesion in PAN involves mitochondrial dysfunction by mtDNA depletion.

Pathogenesis of Progression – Transdifferentiation

W-PO20042

DEVELOPMENTAL TRANSCRIPTION FACTOR SLUG PLAYS A PIVOTAL ROLE IN TUBULAR EPITHELIAL-MESENCHYMAL TRANSITION

TOSHIAKI MONKAWA[1], JUN YOSHINO[2], MIHOKO TSUJI[2], MATSUHIKO HAYASHI[2], TAKAO SARUTA[2]

Department of Internal Medicine, Keio University School of Medicine[1], Keio University School of Medicine[2]

Recently, tubular epithelial-mesenchymal transition (EMT) has emerged as a major pathway leading to renal interstitial fibrosis in diseased kidney. However, the underlying mechanism that governs this process remains largely unknown. The zinc finger protein Slug, a member of the Snail family of transcriptional repressors, plays a crucial role in the EMT pathway during embryonic development and several disease states. This study was conducted to examine the expression of slug in the fibrotic kidney and investigate the function in tubular EMT. First, we investigated the distribution of slug mRNA in various tissues of adult rat. Slug was expressed at relative high level in lung, heart and liver. In kidney, significant slug mRNA expression was observed in embryonic kidney, compared to the low basal levels in adult kidney. Immunohistochemical analysis confirmed that nuclear staining of slug protein was detected predominantly in mesenchymal cells of metanephros, whereas slug staining was virtually absent in adult kidney. Next, we examined the expression of Slug in the fibrotic kidney induced by unilateral ureteral obstruction. Real-time PCR analysis revealed that Slug mRNA expression was significantly increased after ureteral ligation. Immunofluorescence staining demonstrated that the induction of Slug protein was evident in tubular epithelial cells and interstitial fibroblasts in obstructed kidney. Interestingly, some tubular cells expressed both Slug and vimentin, a phenotypic marker of mesenchyme. To elucidate the involvement of slug to tubular EMT, cultured renal epithelial (MCT) cells were stimulated with Transforming growth factor (TGF) [beta]1. TGF-beta1 induced slug mRNA expression in renal tubular epithelial cells in a time- and dose-dependent manner, and MCT cells suppressed E-cadherin expression. These findings suggest that transcriptional repressor Slug is involved in tubular EMT pathway and likely plays a crucial role in the pathogenesis of chronic renal fibrosis.

W-PO20043

ROLE OF CONNECTIVE TISSUE GROWTH FACTOR IN THE TRANSDIFFERENTIATION OF RENAL EPITHELIAL CELLS INDUCED BY TRANSFORMING GROWTH FACTOR-β

HAI-YAN ZHANG[1], YOU-JI LI[1], YAO-ZHONG KONG[2], XUE-QING YU[1]

Department of Nephrology, First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China[1]; The First Hospital of Foshan, Guangdong province, China[2]

Objective: To investigate the role of connective tissue growth factor in transforming growth factor-β-induced tubular-epithelial myofibroblasts tnansdifferentiation.

Methods: NRK52E cells was divided into several groups. The morphological changes were observed by light and electron microscopy (scaning and transmission). Cell immunohistochemistry was used to detect the expression of cytokeratin-18 and α-SMA. RT-PCR and Western-blot were used to detect the expression of collagen type I.

Results: Cultured with 10 ng/ml TGF-β_1 for 3 days, part of the NRK52E cells developed elongated shape, loss of microvilli and apical-basal polarity, and appeared bundles of actin microfilaments. The addition of 10 μg/ml TGF-β_1 neutralizing-antibody and 30 μg/ml CTGF antisense oligonucleotides (ASON) almost completely blocked the morphological changes induced by TGF-β_1, only a little hypertrophy was observed. Cultured with 10 ng/ml TGF-β_1 for 3 days, the expression of cytokeratin-18 significantly decreased, α-SMA and collagen type I significantly increased. Treated with neutralizing-antibody, the expression of cytokeratin-18, α-SMA and collagen type I were almost restored. Changes induced by TGF-β_1 couldn't be restored by CTGF sense oligonucleotides.

Conclusion: It is demonstrated that CTGF as a TGF-β downstream cytokine mediates tubular-epithelial myofibroblasts tnansdifferentiation.

W-PO20044

CYCLOSPORINE A INDUCES EPITHELIAL MESENCHYMAL TRANSITION IN MURINE TUBULAR EPITHELIAL CELL LINES MAINLY BUT NOT EXCLUSIVELY VIA INDUCTION OF TRANSFORMING GROWTH FACTOR (TGF-β1)

FRANK STRUTZ[1], KATRIN NIEDERKLEINE[1], GERHARD ANTON MUELLER[1], ALBERTO ORTIZ[2]

Nephrology and Rheumatology, Georg-August-University Medical Center, Germany[1]; Nephrology, Universidad Autonoma de Madrid, Fundacion Jiminez Diaz, Spain[2]

Chronic therapy with calcineurin inhibitors is often limited due to induction of tubulointerstitial fibrosis. Cyclosporine A (CsA) is known to stimulate fibroblast proliferation and induce extracellular matrix (ECM) production. Recently, epithelial mesenchymal transition (EMT) has been described as a novel mechanism in renal fibrogenesis. We postulated that CSA may induce EMT in tubular epithelial cells and examined the potential mechanisms of that process. All experiments were conducted in NP1 and MCT tubular epithelial cells lines. CsA inhibited proliferation dose and time dependently. Epithelial markers such as E-cadherin were downregulated whereas mesenchymal marker expression such as FSP-1, vimentin and alpha smooth muscle actin was robustly induced. Moreover, CSA induced synthesis of collagen type I (up to 4.8 ± 0.4 ng/ml vs 1.9 ± 0.17 ng/ml in controls) and fibronectin as evaluated by immunoblots and ELISA. Matrix metalloproteinases 2 and 9 were induced up to 1.5-fold. As CSA induced TGF-β1 in both tubular epithelial cell lines by quantitative PCR up to 5-fold, the effects of a neutralizing antibody as well as siRNA for TGF-β1 were evaluated. Both studies demonstrated that the main effects of CSA on EMT are mediated via TGF-β1 although not all effects were neutralized by inhibition of the profibrotic cytokine. Whereas neutralization of FGF-2 did have only minor effects on CSA induced fibronectin synthesis, combined inhibition of TGF-β1 and FGF-2 prevented the effects of CSA completely. In conclusion, the results of this study indicate that CSA may induce EMT in murine tubular epithelial cells mainly via induction of TGF-β1. This mechanism may contribute critically to the chronic tubulointerstitial injury induced by CSA.

Pathogenesis of Progression – Leucocytes

W-PO20045

TACROLIMUS AMELIORATES LATE CONSEQUENCES OF RENAL ISCHEMIC INJURY IN HYPERTENSIVE RATS: REDUCTION OF LEUKOCYTE INFILTRATION AND RANTES EXPRESSION

ONDREJ VIKLICKY[1], JAN RAJNOCH[2], SILVIE BLOUDICKOVA[2], ALENA LODEREROVA[2], EVA HONSOVA[2], ATTILA SZABO[3], ADAM VANNAY[3], STEFAN VITKO[2]

Dept. of Nephrology, Transplant Center, Institute for Clinical and Experimental Medicine, Prague, Czech Republic[1], IKEM[2], Semmelweis University, Budapest[3]

Background: A role of infiltrating leukocytes in kidney disease progression has been discussed. In this study we evaluated the long-term effect of immunosuppressant tacrolimus (TAC) in a model of accelerated renal injury where high-renin hypertension aggravates functional and morphological changes induced by I/R.

Methods: 30 anesthetized uninephrectomised hypertensive transgenic (m-REN-2)-27 rats received clip on renal pedicle for 45 minutes and were treated with TAC (0.10 mg/kg/day i.m.; n = 10), enalapril (ENA, 50 mg/L in the drinking water, n = 10) or placebo (PLA, n = 10) for 12 weeks. 4 animals were sham operated and not treated (Sham). Proteinuria and blood pressure were evaluated throughout and kidneys were harvested for morphological, immunohistochemical (CD5+, CD8+ lymphocytes and ED-1+ macrophages) and RT-PCR analysis (RANTES) at the end of the experiment.

Results: At the end of the study, TAC-treated rats had lower proteinuria as compared to PLA-treated rats (41.1 ± 21.5 vs. 126.7 ± 35.8; $p < 0.01$; ENA: 19.6 ± 4.5, Sham: 28.1 ± 1.3 mg/day) and reduced extent of glomerulosclerosis (4.4 ± 2.3 vs. 20.9 ± 10.9; $p < 0.01$; ENA: 2.7 ± 0.9, Sham: 5.1 ± 1.3%). The PLA- and TAC-treated and Sham operated rats remained hypertensive throughout the experiment and hypertension was reduced in ENA-treated animals. TAC-treated rats had lower CD4+ (54.3 ± 7.7 vs. 125.3 ± 42.8; $p < 0.01$; ENA: 42.37 ± 11.4, Sham 84.9 ± 6.1 cells/field of view), CD5+ (6.8 ± 2.5 vs. 17.1 ± 2.9; $p < 0.01$; ENA: 6.3 ± 2.7, Sham 25.1 ± 2.1 cells/field of view), CD8+ (12.2 ± 2.2 vs. 37.8 ± 14.3; $p < 0.01$; ENA: 13.4 ± 2.0; Sham: 31.8 ± 7.6 cells/field of view) lymphocytes infiltration and ED-1 macrophages infiltration (6.0 ± 1.1 vs. 16.7 ± 4.1; $p < 0.01$; ENA: 4.0 ± 1.2, Sham 12.2 ± 4.6 cells/field of view) than PLA-treated rats. Similarly, TAC-treated rats had lower mRNA RANTES (CCL5)/GAPDH ratio than PLA-treated animals (3.1 ± 0.7 vs. 4.9 ± 1.8; $p < 0.05$; ENA: 2.2 ± 0.6, Sham 4.8 ± 1.3).
Conclusion: Tacrolimus treatment ameliorated late functional and morphological changes induced by I/R injury in hypertensive transgenic rats. Our observation thus supports the hypothesis about the key role of lymphocytes and macrophages in renal injury caused by ischemia and hypertension.

W-PO20046

THE ROLE OF THYMOSIN BETA 4 IN RENAL FIBROSIS

MIHOKO TSUJI[1], TOSHIAKI MONKAWA[1], JUN YOSHINO[1], MATSUHIKO HAYASHI[1], TAKAO SARUTA[1]
Keio University School of Medicine[1]

To search for genes playing important roles in progression of renal diseases, we compared gene expression profiles between an irreversible model and a reversible model of anti-Thy-1 glomerulonephritis using a microarray technology. A single intravenous injection of anti-Thy-1 monoclonal antibody (MoAb) 1-22-3 is known to cause a reversible mesangial proliferative glomerulonephritis. However, MoAb 1-22-3 injection followed by unilateral nephrectomy leads to progressive glomerulosclerosis with an irreversible course. Among 4854 rat genes on the microarray slide, 191 genes were differentially expressed. The differentially expressed genes were classified into 7 clusters based on their expression patterns. One of the clusters included genes the expression of which was markedly upregulated in the irreversible model. The expression levels of the genes belonging to the cluster were low in normal kidney and increased along with disease peaked at day 14, and the upregulation was more pronounced in the irreversible model than the reversible model. This cluster included collagen type1, laminin, Osteopontin, Kidney injury molecule-1 (KIM-1), and Thymosin b10. Thymosin b10, and its homologue, Thymosin b4 are actin-binding peptides, and are known to be an important mediator of cell proliferation, migration, differentiation, and regeneration. Thymosin b4 and b10 were expressed in fibrotic interstitium of the anti-Thy-1 nephritis rat kidney and the unilatelaly ureteral obstructed kidney. The macrophages positive for ED-1 were found to express Thymosin b4. In cultured human monocyte THP-1 cells, expression for Thymosin b4 and b10 were upregulated along with the activation. Thymosin b4 and b10 may play an important role in renal fibrosis via macrophage activation.

W-PO20047

PERIPHERAL B LYMPHOCYTE β1,3 GALACTOSYLTRANSFERASE EXPRESSION IN IGA NEPHROPATHY

WEI QIN[1], QIN ZHOU[2], LICHUAN YANG[1], ZI LI[1], BAIHAI SU[1], JUNMING FAN[1]
Department of nephrology West China Hospital, Sichuan University, Chengdu 610041, Sichuan, P.R.China[1], State Key Laboratory Of Biotherapy For Human Diseases, Cheng Du,China[2]

Purpose: Serum IgA_1 aberrant O-glycosylation is presumed to be the main pathogenesis of IgA nephropahty. β1,3 galactosyltransferase is the main enzyme participate in the glycosylation process. To investigate the underlying mechanism of aberrant glycosylation, β1,3 galactosyltransferase (C1GALT1 and C1GALT2) expression in IgA nephropthy, its relationship to clinical manifestation, pathological grading and IgA galactosylation level were studied.
Methods: Forty patients with IgAN, 32 patients with non-IgAN glomerulonephritis and 25 normal controls were included in this study. Peripheral B lymphocytes were isolated using CD19 magnetic beads, then β1,3 galactosyltransferase (C1GALT1 and C1GALT2) expressions were quantitatively measured using real-time RT-PCR. Serum IgA level and its galactosylation level were measured using ELISA and VV lectin binding method. Relationship analysis was performed between the clinical features (severe proteinuria, hypertension, renal dysfunction, gross hematuria) pathological grading and β1,3 galactosyltransferase expression.
Results: B lymphocyte C1GALT2 gene expression was significant decrease in IgAN patients than normal controls and non-IgAN patients ($p < 0.05$), but not in C1GALT1 gene. The C1GALT2 expression showed a negative correlation with VV lectin binding results in IgAN patients ($r = -0.85$, $p < 0.05$) but not in controls. Statistical analysis indicated that C1GALT2 expression was positively related to severe proteinuria ($P < 0.01$), hypertension, renal dysfunction, pathological grading ($p < 0.05$); but not related to gross hematuria ($p > 0.05$).
Conclusion: These data indicated that the altered IgA O-galactosylation in IgAN results from a B lymphocyte downregulation of β1,3 galactosyltransferase gene expression (C1GALT2), which is closely related to clinical manifestations of the disease. This downregulation may be a fundamental pathogenic abnormality in IgAN.

W-PO20048

COGNATE IMMUNE RESPONSES DO NOT ACCOUNT FOR THE INFLAMMATORY RESPONSE IN MURINE FSGS

LUKAS K. KAIRAITIS[1], YIPING WANG[1], YUET-CHING TAY[1], DAVID CH HARRIS[1]
Centre for Transplantation and Renal Research, The University of Sydney at Westmead Millenium Institute, Australia[1]

Background: Although inflammatory effector cells are thought to play a role in the progression of renal injury, the role of cognate pathways of activation of these cells is unknown. We have previously shown that blockade of CD40-CD40L interaction is effective at reducing the severity of murine adriamycin nephrosis (AN), although it remains unclear if this effect is due to interruption of the effects of this pathway on innate or cognate pathways.
Methods: The role of cognate immune pathways in AN was further studied by blockade of the CD28-B7 pathway responsible for primary cognate T cell responses using a CTLA4-Fc fusion protein. The severity of AN at day 42 after adriamycin was compared among mice treated with adriamycin (9.6 mg/kg, n = 8), mice treated with 500 mg of CTLA4-Fc fusion protein prior to adriamycin (n = 8) and control animals (n = 8). Disease severity was compared among groups by functional markers, quantitative histology and immunohistochemical quantitation of cortical inflammatory cells.
Results: Significant adriamycin nephrosis developed in both adriamycin-treated groups, with significantly worse parameters for glomerulosclerosis, tubular atrophy and interstitial expansion (all $p < 0.01$). Similarly, cortical inflammatory cells (CD4+ cells, CD8+ cells and macrophages) were significantly more numerous than controls (all $p < 0.01$). There was no detectable difference in the degree of cortical inflammation or renal injury in animals from the CTLA4 + adriamycin or adriamycin-alone groups.
Conclusion: These results suggest that *primary* cognate T cell responses (which are dependent upon CD28-B7 interactions) do not significantly contribute to the pathogenesis of renal inflammation or injury in murine adriamycin nephrosis.

Pathogenesis Of Progression – Endothelial Factors

W-PO20049

INTRARENAL, PLASMATIC AND URINARY TISSUE FACTOR (TF) AND ITS INHIBITOR (TFPI) IN CHRONIC GLOMERULONEPHRITIS AND HEALTHY SUBJECTS

BEATA NAUMNIK[1], JACEK BORAWSKI[2], LECH CHYCZEWSKI[2], OKSANA KOVALCHUK[2], MICHAŁ MYŚLIWIEC[1]
Dept. of Nephrology and Transplantology with Dialysis Unit, Medical University of Bia_ystok, Poland[1], Dept. of Clinical Molecular Biology, Medical University of Bia_ystok, Poland[2]

Tissue factor (TF) – the most potent trigger of coagulation and emerging anti-apoptotic, proliferative and angiogenic factor, along with its principal inhibitor (Tissue factor pathway inhibitor, TFPI) are supposed to be involved in chronic glomerulonephritis (GN). We examined for the first time: 1) expression of TF, TFPI 1 and TFPI 2 antigens (immunoperoxidase histochemistry) in kidney biopsy specimens; 2) plasma and urine levels of TF and total TFPI (pre-biopsy, ELISA). The study enrolled 30 chronic GN patients [IgAN (n = 8), FSGS (n = 10), membrano-proliferative GN (n = 7), membranous GN (n = 4), and fibrillary GN (n = 1)] and 18 healthy subjects. Biopsy control consisted of 2 sections from normal areas of nephrectomy specimens. Only plasma and urinary TF were markedly higher in patients than in controls.

In normal kidney specimens TF, TFPI 1 and TFPI 2 were undetectable in glomeruli but were observed in interstitial microvessels. In diseased kidneys, TF was strongly expressed in glomeruli but was undetectable in tubules. In contrast, staining for both TFPI variants were observed in glomeruli and tubules. Neither plasma nor urinary levels of the markers correlated with the intensity of TF and TFPI 1/2 expression in biopsy specimens. Interestingly, urinary TF was significantly and directly associated with CrCl ($R = 0.489$, $P = 0.006$). It was also positively associated with urinary TFPI ($R = 0.388$, $P = 0.034$), and tended to be lower in proliferative vs non-proliferative GN [83 (0–617) pg/ml vs 281 (10–805) pg/ml; $P = 0.06$].

In conclusion, intrarenal TF/TFPI system is deeply abnormal in chronic GN and may be responsible for its development and/or progression. Plasma and urinary concentrations of TF and TFPI do not reflect genuine activity of the disease, likely due to confounding effect of kidney insufficiency. Urinary TFPI measurement may be helpful in initial identification of proliferative GN.

W-PO20050

AN ATTEMPT OF EVALUATION OF INFLAMMATORY PROCESS ACTIVITY ASSESSED BY THE LEVEL OF CYTOKINS IL-6 AND RANTES AND ENDOTHELIAL INJURE IN PATIENTS WITH IGA NEPHROPATHY

BEATA SULIKOWSKA[1], WITOLD DOROSZEWSKI[1], MAGDALENA GRAJEWSKA[1], ANNA STEFAŃSKA[2], GRAŻYNA ODROWÁ8Ż-SYPNIEWSKA[2], DANUTA ROŚĆ[3], JACEK MANITIUS[1]

Dept. of Nephrology, Hypertension and Internal Diseases Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz, Poland[1], Dept. of Clinical Biochemistry Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz, Poland[2], Dept. of Pathophysiology Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz, Poland[3]

The levels of cytokins (Il-6 and RANTES) in serum are indicators of inflammation process, whereas vWF:Ag, t-PA, PAI are the markers of endothelial injure. A proteinuria, lipid and purin metabolism disorder, blood pressure are considered as parameters describing the disease process advancement and determined to the progression of primary nephropathy.

The aim of our study was to find the relations between the inflammation process activity assessed by the level of Il-6 and RANTES concentration and endothelial injure, and other indicators of the renal disease progression during 12 months therapy.

Materials and Methods: 22 patients with IgA nephropathy proven by the renal biopsy, aged 34 ± 8.7 yrs, with creatinine clearance: 101.5 ± 33.10 ml/min. The following parameters: Il-6, RANTES, vWF:Ag, t-PA, PAI, total cholesterol (TCH), LDL, HDL, triglycerides (TG), uric acid (UA), 24-hour urinary extrection of N-acetyloglucosaminidase (NAG) and protein were assessed before (0) and after 12 months. All were treated with ACEi, 8 pts. steroids and statins.

Results: (average ± SD)

Parameter	(0)	(12)	p
SBP (mmHg)	135.68 ± 13.3	127.73 ± 18.24	<0.05
DBP (mmHg)	81.36 ± 8.48	75.68 ± 10.38	<0.05
RANTES (ng/ml)	92.05 ± 35.22	67.23 ± 25.49	<0.01
I1-6 (pg/ml)	21.57 ± 26.64	8.55 ± 13.92	<0.01
24 hrs protein (g)	2.78 ± 2.01	1.43 ± 1.66	<0.01
TG (mg/dl)	152.6 ± 99.2	148.6 ± 125.8	ns
TCH (mg/dl)	245.0 ± 83.7	210.5 ± 75.8	ns
LDL (mg/dl)	153.9 ± 61.4	133.4 ± 56.9	<0.05
HDL (mg/dl)	50.8 ± 9.3	53.1 ± 7.3	ns
UA (mg/dl)	6.06 ± 1.4	5.25 ± 1.1	<0.05
NAG (U/g cr)	6.33 ± 3.63	5.37 ± 2.61	<0.05
vWF:Ag	118.8 ± 14.5	100.9 ± 25.1	<0.01
t-PA	8.89 ± 5.04	5.51 ± 4.35	<0.05
PAI	20.18 ± 7.08	16.72 ± 10.36	ns

Significant correlations were found between Il-6 vs UA and 24 hrs protein ($r = 0.6$ $p < 0.01$; $r = 0.91$ $p < 0.01$ resp.), RANTES vs UA, 24 hrs protein and DBP ($r = 0.53$ $p < 0.05$; $r = 0.78$ $p < 0.01$ i $r = 0.63$ $p < 0.05$ resp.), changed levels (D) during therapy of Il-6 and D RANTES vs 24 hrs protein ($r = 0.5$ $p < 0.01$ and $r = 0.49$ $p < 0.01$ resp.), D vWF:Ag and D t-PA vs 24 hrs protein ($r = 0.43$ and 0.39, $p < 0.05$ resp.).

Conclusions: 1. Inflammation process in the course of IgA nephropathy seems to be related with 24 hrs protein, serum uric acid and DBP.

2. The used therapy lowered the extant of inflammatory process and endothelial injure, and it was seen in decrease of proteinuria.

W-PO20051

DIRECT VISUALIZATION OF CORONARY ENDOTHELIAL DYSFUNCTION IN EARLY STAGE CHRONIC RENAL FAILURE (CRF)

SATORU TATEMATSU[1], KOICHI HAYASHI[1], SHU WAKINO[1], ICHIRO TAKAMATSU[1], NAOKI SUGANO[1], TAKESHI KANDA[1], KOICHIRO HOMMA[1], KYOKO YOSHIOKA[1], KAZUHIRO HASEGAWA[1], TAKAO SARUTA[1]

Department of Internal Medicine, School of Medicine, Keio University[1]

Purpose: We previously demonstrated that coronary endothelial dysfunction developed in dogs with 5/6-nephrectomy (serum creatinine, 1.60 ± 0.09 mg/dL). It has not been elucidated however whether coronary endothelial function is impaired in earlier stage CRF. In the present study, we examined the coronary endothelial function in early stage CRF by visualization of coronary microcirculation with charge-coupled device (CCD) camera.

Methods: Mongrel dogs were divided into three groups; sham-operation, 1/2-nephrectomy or 5/6-nephrectomy. Four weeks after the operation, coronary blood flow (CBF) responses to acetylcholine (ACh) and nitroprusside (SNP) were evaluated with an ultrasonic flow probe placed at the left circumflex coronary artery. Simultaneously, the diameter of coronary arterioles was measured using CCD camera microscope. Finally, whether GFR or asymmetrical dimethylarginine (ADMA; intrinsic NOS inhibitor) was better correlated with the degree of coronary dysfunction was also evaluated.

Results: Renal ablation reduced GFR (sham; 76.2 ± 6.7, 1/2-nephrecomy; 38.4 ± 3.8, 5/6-nephrecomy; 19.3 ± 3.0 ml/min) and elevated plasma ADMA levels (sham; 2.6 ± 0.2, 1/2-nephrecomy; 3.1 ± 0.5, 5/6-nephrecomy; 3.5 ± 0.3 mmol/L). CBF responses to ACh were impaired in 1/2-nephrecomy group and 5/6-nephrecomy group; the ACh-induced increments in CBF was inversely correlated with plasma ADMA ($R^2 = 0.396$), but not with GFR. In contrast, the CBF responses to SNP did not differ among three groups. Direct visualization of ACh-induced vasodilation of coronary arterioles paralleled the changes in CBF.

Conclusions: Endothelial function of coronary arterioles is impaired even in early stage of CRF, which is associated with the level of ADMA. Thus, early detection of renal impairment and therapeutic modification of plasma ADMA levels would modify the incidence of cardiovascular events in renal disease.

W-PO20052

DIETARY DOSE OF NITRITE ATTENUATES L-NAME-INDUCED RENAL INJURY IN RATS

TOSHIAKI TAMAKI[1], MASUMI OKAMOTO[1], YASUHISA KANEMATSU[1], YUKI IZAWA[1], SHOJI KAGAMI[1], SHUJI KONDO[1], MASANORI YOSHIZUMI[1], KOICHIRO TSUCHIYA[2]

The University of Tokushima School of Medicine[1], Graduate School of Pharmaceutical Sciences, University of Tokushima[2]

We have reported that oral nitrite increases circulating nitric oxide (NO) and have an important physiological role in protecting cardiovascular diseases (Am J Physiol Heart Circ Physiol in press) and the nitrite can be an alternative source of NO in ischemic kidney (Am J Physiol Renal Physiol 288:F182–187, 2005). In this study, we examined the effect of chronic administration of dietary dose of nitrite on the renal injury induced by chronic L-NAME intake in rats. The animals was administered tap water containing L-NAME (1 g/L) or L-NAME + nitrite (0.1 mg/L, 1 mg/L, 10 mg/L, 100 mg/L) for eight weeks. We evaluated the blood NO levels as a hemoglobin-NO adducts (Hb-NO) using electron paramagnetic resonance (EPR) method. The Hb-NO signal was obtained by subtracting the EPR spectrum of blood of control rats.

1) Chronic administration of L-NAME for 8 weeks induced renal injury in rats and reduced the Hb-NO level in blood (control; 38.8 + 8.9 vs L-NAME; 6.0 + 3.1 arbitrary units). Renal damages were characterized by an increase in the number of glomeruli with protein droplets in visceral epithelial cells, the occurrence of glomeruli with ischemic change and tubulointerstitial changes. 2) Co-administration of nitrite (1 mg/L, 10 mg/L, 100 mg/L) with L-NAME increased

the Hb-NO signal in blood (L-NAME; 6.0 + 3.1, 0.1 mg/L; 11 + 2.9, 1 mg/L; 30 + 3.9*, 10 mg/L; 36 + 6.2*, 100 mg/L; 31 + 1.6*, arbitrary units, *$p < 0.05$ vs L-NAME). Co-administration of nitrite with L-NAME for 8 weeks attenuated L-NAME-induced renal histrogical changes.
These results suggest that dietary dose of nitrite can be an alternative source of NO in vivo and have an important physiological role in protecting renal damages.

Pathogenesis of Progression – Matrix And Fibrosis

W-PO20053

THE INDUCIBLE BRADYKININ B1 RECEPTOR: A PUTATIVE TARGET IN RENAL TUBULOINTERSTITIAL FIBROSIS

__JEAN-LOUP BASCANDS__[1], JULIEN GONZALES[1], LAURE ESPOSITO[1], DENIS CALISE[2], ERIC NEAU[3], JOOST P. SCHANSTRA[1]
Inserm U388, IFR 31, Institut Louis Bugnard, BP 84225, 31432 Toulouse Cedex 4, France[1], Service of microsurgery, IFR 31, Institut Louis Bugnard, BP 84225, 31432 Toulouse Cedex 4, France[2]

It is now well admitted that angiotensin converting enzyme (ACE) inhibitors reduce the progression of fibrotic renal disease both in men and animal models. Inhibition of ACE decreases angiotensin II formation and increases bradykinin concentrations. Under ACE inhibitor treatment not only bradykinin is accumulating but also its metabolite des-Arg^9-bradykinin, which is the natural agonist of the bradykinin B1 receptor. The B1 receptor is, in contrast to the B2 receptor, poorly expressed under physiological conditions but highly over-expressed in a variety of inflammatory pathologies.
Using a model of accelerated renal tubulointerstitial fibrosis induced by unilateral ureteral obstruction (UUO), we have previously reported that bradykinin B2 receptor activation reduced renal tubulointerstitial fibrosis *in vivo* (Schanstra et al., *J. Clin. Invest.* 110:371). To fully understand the role of bradykinin and its receptors in the development of renal interstitial fibrosis we have investigated the role of the bradykinin B1 receptor.
In wild type mice, 5 days of UUO induces a 2.3 fold over-expression of bradykinin B1 receptor mRNA in the obstructed kidney. In the next experiment we compared the development of renal fibrosis in wild type and B1 receptor knockout mice. 5 days of obstruction showed reduced interstitial macrophage infiltration and myofibroblasts accumulation in B1 receptor knockout mice compared to wild type mice. This was accompanied by lower UUO-induced tubulointerstitial fibrosis in B1 receptor knockout mice.
These data strongly suggest a profibrotic role for the bradykinin B1 receptor and suggest that it might be a new therapeutic target to slow down the progression of renal tubulointerstitial fibrosis.

W-PO20054

FETUIN-A MODULATES MONOCYTE INFLUX AND TGF-BETA EXPRESSION IN EXPERIMENTAL RENAL FIBROSIS

__RALF WESTENFELD__[1], THILO KRÜGER[1], VINCENT BRANDENBURG[1], RALF SMEETS[2], CORA SCHÄFER[2], SABRINA STOJANOW[1], JÜRGEN FLOEGE[1], WILLI JAHNEN-DECHENT[2], MARKUS KETTELER[1]
Nephrology & Immunology, University Hospital Aachen, Germany[1], IZKF BioMAT, University Hospital Aachen, Germany[2]

Infiltration of blood mononuclear cells, tubulointerstitial scarring and glomerulosclerosis characterize the final pathway towards end-stage renal disease. The multifunctional calcium-regulatory protein fetuin-A has been implicated as an endogenous antagonist of the profibrotic growth factor TGF-b, acting as a circulating decoy receptor, and as a modulator of macrophage activation. Notably, in proteinuric states fetuin-A deficiency develops due to 'fetuinuria'. In the present study we investigated macrophage influx, TGF-b induction and matrix accumulation in fetuin-A deficient (fet$^{-/-}$) and wild-type (WT) mice with unilateral ureteral obstruction.
Mice (n = 42) were investigated on days 7 and 30 after ureteral ligation. Single cell suspensions from whole kidneys were stained for F4/80, MHC-II and intracellular iNOS, and subjected to FACS analysis. Realtime PCR with an absolute quantitative approach was used to determine renal TGF-b and fibronectin mRNA expression.
Fetuin-A deficiency did not affect renal histology or macrophage influx in non-obstructed kidneys. On day 7 after ureteral obstruction we detected a significantly increased macrophage influx into kidneys of fet$^{-/-}$ mice (F4/80 positive cells – fet$^{-/-}$: 22.3 ± 4.9 vs. WT: 13.1 ± 5.1*; $p < 0.05$), while the macrophage activation state was not different between groups as judged by similar MHC-II and intracellular iNOS expression per F4/80 positive cell, respectively. The more intense macrophage influx in fet$^{-/-}$ mice was accompanied by a significantly higher TGF-b and fibronectin mRNA expression on day 7 after ureteral obstruction (TGF-b: 5254 ± 797 vs. 845 + 134* copies/1Mio copies 18S RNA; fibronectin 75 ± 18 vs. 32 ± 9* copies/1Mio copies 18S RNA; *$p < 0.05$).
In fet$^{-/-}$ mice we observed an upregulated renal TGF-b expression after unilateral ureteral ligation, possibly due to the loss of TGF-b-antagonistic activity and subsequent AP1-dependent TGF-b autoinduction. Fetuin-A deficiency or the resulting increased TGF-b bioactivity significantly enhanced macrophage influx in the initial phase of experimental renal fibrosis as well as downstream expression of matrix molecules (e.g. fibronectin).

W-PO20055

THE EFFECT OF TUBULAR EPITHELIAL CELLS ACTIVATED BY ALDOSTERONE ON RENAL INTERSTITIAL FIBROBLASTS IN CO-CULTURE SYSTEM

__YANG LUO__[1], YIPU CHEN[1]
Center of Nephrology, China-Japan Friendship Hospital, Beijing, 100029 P. R. China[1]

Background: We have recently reported that local aldosterone (ALDO) could be synthesized by human proximal tubular epithelial cell lines (HKC) under the stimulation of endothelin-1 (ET-1) in vitro. This study was designed to observe the effect of tubular epithelial cells activated by aldosterone on renal interstitial fibroblasts in co-culture system.
Methods: HKC were stimulated with ALDO at different concentrations and times, RT-PCR and ELISA were performed to detect TGF-b1 expression; (2) HKC were co-stimulated with ET-1 and spironolactone at different concentrations and times to evaluate the influence of endogenous ALDO on TGF-b1 expression; (3) HKC which were activated by ALDO, and hRIFs were co-cultured with or without anti-TGF-b1 antibody in the media, the production of collegen I (Col-I) in the cell layer of hRIFs was detected by ELISA.
Results: The expression of TGF-b1 increased in a dose- (0, 10^{-11}, 10^{-9} 10^{-7} mol/L) and time- (0, 12, 24, 48 h) dependent manner in both mRNA and protein levels after stimulation of ALDO ($P < 0.05$); The reduction of TGF-b1 had a dose- (0, 10^{-11}, 10^{-9} 10^{-7} mol/L) and time (0, 12, 24, 48 h) dependent manner with the spironolatone ($P < 0.05$); The production of Col-I increased significantly comparing with that of the control group after co-culture ($P < 0.01$), anti-TGF-b1antibody could partially inhibit this effect ($P < 0.05$).
Conclusion: The expression of TGF-b1 in HKC can not only be upregulated by exogenous ALDO, but also can be upregualted by endogenous ALDO in an autocrine manner. HKC activated by ALDO can promote the synthesis of Col-I in hRIFs by a 'cross talking' way, this effect is partially mediated by TGF-b1.

W-PO20056

THE EFFECTS OF ANGIOTENSIN II RECEPTOR ANTAGONIST AND ENDOTHELIN RECEPTOR ANTOGONIST ON RENAL TUBULOINTERSTITIAL FIBROSIS OF CHRONIC ARISTOLOCHIC ACID NEPHROPATHY

__CONG ZHANG__[1], YIPU CHEN[1], HONGRUI DONG[1]
Center of Nephrology, China-Japan Friendship Hospital, Beijing, 100029 P. R. China[1]

Objective: To investigate the effects of angiotensin II receptor antagonist (valsartan) and endothelin receptor antogonist (bosentan) on the renal injury of chronic aristolochic acid nephropathy (CAAN) rats.
Methods: To establish a rat model of CAAN with aristolochia manshuriensis Kom by gavage intermittently, and divide them into model group, valsartan group (30 mg/kg/d) and bosentan group (100 mg/kg/d). Control group (CTR) received only tap water. Each group consisted of 6 rats. Assessments of proteinuria, urinary b2 microgloubumin and serum creatinine (Scr) had been done by the end of 1st, 4th, 8th, 12th and 16th weeks. Then the rats were sacrificed and histological analysis of kidney was performed. The mRNA and the protein expressions of transforming growth factor-b1 (TGF-b1), connective tissue growth factor

(CTGF), Plasminogen activator inhibitor-1 (PAI-1), Tissue inhibitor of metalloproteinase-1 (TIMP-1) and Type I Collagen (Col I) in kidney tissue were semi-quantitatively determined by RT-PCR and immunohistochemical staining respectively.

Results: Compared with CTR, proteinuria, urinary b2 microgloubumin and Scr were significantly increased in the CAAN group ($P < 0.05$). Relative area of interstitial fibrosis was also significantly enlarged ($P < 0.01$). The expressions of TGF-b1, CTGF, PAI-1, TIMP-1 and Col I mRNA and protein were up-regulated ($P < 0.01$). After intervention with valsartan and bosentan, the up-regulation of the above mentioned parameters were all markedly reduced ($P < 0.05$). But there was no different between valsartan group and bosentan group.

Conclusion: Valsartan and bosentan might ameliorate renal tubulointerstitial fibrosis, probably through inhibiting the accumulation of extracellular matrix in renal interstitium.

W-PO20057

FUNCTIONAL SIGNIFICANCE OF PI3 KINASE AND MTOR IN THE REGULATION OF RENAL FIBROBLAST FUNCTION

CATHERINE WINBANKS[1], TIM HEWITSON[1], LAUREN GRIMWOOD[1], ANNA GASSER[1], IAN DARBY[2], GAVIN BECKER[1]
Royal Melbourne Hospital[1], RMIT University[2]

The recruitment of fibroblasts is an essential part in the pathogenesis of tubulointerstitial fibrosis. Despite their significance their regulation remains poorly understood. In this study we investigated the role of specific PI3 kinase/mTOR pathway inhibitors in the regulation of renal fibroblast function.

Rat renal fibroblasts were propagated from kidney tissue post-ureteric obstruction. Specific inhibitors upstream (LY294002) and downstream (rapamycin derivative RAD) of Akt phosphorylation were used to examine the effects on cell kinetics (changes in thymidine incorporation and cell number) and myofibroblast differentiation (cytochemistry for alpha smooth muscle actin; SMA). A mitochondrial activity test (MTT dye reduction) and propidium iodide staining were used to substantiate that effects were independent of cell toxicity.

As expected, western blotting confirmed that LY294002 (10 μM) but not RAD prevented phosphorylation of Akt. RAD (10, 100, 200 nM) inhibited serum induced mitogenesis by 53 ± 7%, 50 ± 9% and 42 ± 7% respectively (all $p < 0.01$ vs basal) with 100, 200 nM RAD decreasing population growth over 5 days by 63 ± 10%, and 65 ± 14% respectively (both $p < 0.001$ vs basal). Likewise 10 μM LY294002 decreased mitogenesis by 32 ± 12% and population growth by 44 ± 8% (both $p < 0.05$ vs basal). Treatment with 100, 200 nM RAD and LY294002 increased the proportion of cells staining for SMA, and therefore myofibroblasts, by 18%, 31% and 20% respectively (all $p < 0.05$ vs basal, $n = 3$ each group). These effects were independent of cell toxicity.

In conclusion, the regulation of fibroblast activity in vitro involves the PI3 kinase pathway and mTOR. Two specific inhibitors have been shown to decrease cell proliferation but interestingly increase myofibroblast differentiation. These results highlight the potential significance of these pathways in renal fibrogenesis.

W-PO20058

ALL-TRANS RETINOIC ACID MARKEDLY HALTED RENAL DAMAGE OF 5/6 RENAL ABLATION RATS

MAN LI[1], AI PENG[1], YONG GU[1], SHANYAN LIN[1]
Nephrology Research Institute and Division of Nephrology, Huashan Hospital, Fudan University, China[1]

Objective: Glomerulosclerosis is a common pathological event to different kidney diseases. Some evidences showed that all trans retinoic acid (atRA) could regress the mesangial cells proliferation and reduce podocytes damage in acute experimental glomerulonephritis, which clued on a hypothesis that atRA halts the development of glomerulosclerosis.

Methods: 5/6 renal ablation rats divided into atRA treated groups (5 mg/kg/d, 10 mg/kg/d and 20 mg/kg/d, $n = 8$) and vehicle group ($n = 8$). Health rats consist of sham-operation group ($n = 8$). Concentrations of atRA in plasma and renal tissues were measured by Reversed Phase High Performance Liquid Chromatography (RP-HPLC). Glomerulosclerosis was evaluated by glomerulosclerosis index system. activator protein-1 (AP-1) mRNA expressions were quantitated by real-time PCR. The level of TGFβ1 was assayed by renal immunohistochemical staining and Western blot.

Results: From 6 to 10 weeks mean artery pressures of all nephroectomic rats treated by atRA became much lower than those of vehicle group, which paralleled to the decreases of ratio of urinary albumin and urinary creatinine. The atRA concentrations either in plasma or renal tissue of atRA treated groups were much higher than those of untreated rats. The ratios of kidney weight and body weight in vehicle group were much higher than that of atRA treated rats ($P < 0.05$). The glomerulosclerosis scores in atRA treatment groups were 120.4 ± 58.7 (5 mg/kg/d), 111.9 ± 52.9 (10 mg/kg/d) and 81.2 ± 32.6 (20 mg/kg/d) respectively which is much lower than that in vehicle group (51.6 ± 62.8, $P < 0.05$). The lower levels of mRNA expression of c-jun and c-fos and TGFβ1 protein expression in renal cortex was observed in atRA treatment groups ($P < 0.05$).

Conclusion: 1) In this present study we proved firstly that atRA delayed the development of chronic glomerular sclerosis, which may be mediated by the depression of AP-1 or TGFβ1 expressions. 2) atRA could be absorbed by rats through gastrointestinal tract and accumulated in remnant kidney.

W-PO20059

OVER-EXPRESSION OF TISSUE TRANSGLUTAMINASE (TTG) IN PROXIMAL TUBULAR EPITHELIAL CELLS (PTEC) AFFECTS EXTRACELLULAR MATRIX (ECM) ACCUMULATION IN VITRO

MARIE FISHER[1], LINGHONG HUANG[1], ZOE HAU[1], MARTIN GRIFFIN[2], MEGUID EL NAHAS[1], TIM JOHNSON[1]
Sheffield Kidney Institute, Sheffield University, UK[1], Aston Academy of Life Science, Aston University, UK[2]

The up-regulation and release of tTg by tubular epithelial cells has previously been associated with the development of tubulo-interstitial scarring. tTg is a calcium dependent enzyme that catalyses the formation of e(g-glutamyl) lysine iso-peptide bonds. We have proposed that this enzyme may contribute to scarring by accelerating matrix deposition and stabilise the ECM against the proteolytic action of enzymes such as matrix metalloproteinases (MMP's).

To address this, we have generated by stable transfection a PTEC cell line that possesses an 11 fold increase in tTg expression (tTg+) compared to wild type (WT) cells. We have used these cells to study the effect of tTg over-expression on the deposition and breakdown properties of the ECM.

In tTg+ PTEC, increased cellular tTg expression leads to a corresponding increase in cell surface tTg expression. 3H proline incorporation into deposited ECM demonstrates that tTg+ PTEC produce matrix with an elevated collagen content, however total ECM levels remain unchanged. Immunofluorescence analysis indicates that changes in collagen composition are primarily due to increased collagen III and IV. Inhibition of MMP activity by treatment with 10 mM Galardin increases collagen deposition in WT and tTg+ ECM over 72 hours. However, the proportional increase is greater in tTg+ ECM (62 ± 0.95 to 70.6 ± 1.19 dpm/mg protein) than in WT ECM (54.1 ± 5.3 to 58 ± 9.3 dpm/mg protein). No significant difference in turnover rates between tTg+ and WT ECM is observed. Treatment of tTg+ ECM with MMP's 1 and 2 reduces the percentage degradation per hour of the ECM from 2.3% to 1.1%.

Altering tTg expression results in qualitative changes to the ECM primarily by accelerating collagen deposition, but also by altered susceptibility of the tubular ECM to decay. This provides strong evidence to support a role of tTg in the stabilisation of the expanding ECM associated with tubulointerstitial scarring.

W-PO20060

'ASSOCIATION OF PIRFENIDONE, LOSARTAN AND MMF INDUCES EFFECTIVE RENOPROTECTION IN AN EXPERIMENTAL MODEL OF CHRONIC PROGRESSIVE RENAL DISEASE'

IRENE L. NORONHA[1], CAMILE ALBA PEREIRA[1], HUMBERTO DELLE[1], DENISE M. A. MALHEIROS[1], SOLOMON MARGOLIN[2], IRENE L. NORONHA[1]
Renal Division, University of Sao Paulo, Brazil[1], Marnac, Inc, USA[2]

Chronic progressive renal disease is characterized by excessive deposition of extracellular matrix leading to the development of fibrosis. The use of angiotensin blockers, such as losartan (LOS), and anti-inflammatory drugs as mycophenolate mofetil (MMF), do not completely block the progression of renal fibrosis. A possible alternative therapeutic approach could be the use of anti-fibrotic drugs, such as pirfenidone. The aim of the present study was to analyze the effect of pirfenidone as monotherapy and also associated with LOS and MMF, in an experimental model of progressive renal disease induced by chronic inhibition of nitric oxide, using L-NAME.

Male Wistar rats were distributed into six groups: **Control** (n = 5), rats receiving only high salt diet (HS, 3.2% Na); **NAME** (n = 8), rats receiving L-NAME (200 mg/L) and HS diet during 30 days to induce progressive renal disease; **LOS** (n = 5), NAME-rats receiving losartan (50 mg/kg/day) in the water; **MMF** (n = 8), NAME-rats receiving MMF (10 mg/kg/day); **PIRF** (n = 10), NAME-rats receiving pirfenidone (chow with 500 mg/kg/day); and **PIRF + LOS + MMF** (n = 10), NAME-rats receiving triple therapy consisting of pirfenidone + losartan + MMF.

The parameters analyzed after thirty days were: body weight (BW), delta body weight (DBW), tail-cuff pressure (TCP), albuminuria ($U_{alb}V$), glomerulosclerosis index (GS), % of collapsed glomeruli (collapsed G), and % of interstitial fibrosis (Int Fibrosis).

Results: mean ± SEM; ^{a} $p < 0.05$ vs Control; ^{b} $p < 0.05$ vs NAME; ^{c} $p < 0.05$ vs LOS

	Control	NAME	LOS	MMF	PIRF	PIRF + LOS + MMF
Initial BW	245 ± 25	226 ± 11	231 ± 25	234 ± 12	254 ± 21	223 ± 13
Final BW	368 ± 31	267 ± 18	330 ± 17	315 ± 11^a	339 ± 16	334 ± 9^a
Δ BW (%)	50%	18%	43%	44%	33%	50%
TCP (mmHg)	140 ± 8	179 ± 3^a	177 ± 0.7^a	177 ± 2^a	171 ± 7^a	171 ± 4^a
$U_{alb}V$ (mg/24 h)	0.6 ± 0.5	83.2 ± 11.8^a	20.0 ± 5.0^{a,b}	34.8 ± 17.3^a	32.0 ± 10.0^{a,b}	11.0 ± 3.0^{a,b}
GS (%)	0.1 ± 0.1	8.3 ± 2.2^a	2.2 ± 0.6^{a,b}	2.6 ± 0.8^{a,b}	2.9 ± 0.9^a	2.0 ± 0.4^{a,b}
Collapsed G (%)	0.2 ± 0.2	9.6 ± 2.5^a	0.7 ± 0.4^b	5.7 ± 3.0	1.6 ± 1.0^b	0.9 ± 0.3^b
Int Fibrosis (%)	0.1 ± 0.0	2.8 ± 0.7^a	0.8 ± 0.1^b	0.7 ± 0.2^b	0.4 ± 0.1^{b,c}	0.4 ± 0.1^{b,c}

After 30 days, NAME-rats developed hypertension, albuminuria, glomerulosclerosis and interstitial fibrosis, with consequent lower body weight gain. Monotherapy with pirfenidone significantly diminished albuminuria, collapsed G, and interstitial fibrosis, similar to LOS and MMF monotherapies. However, the best renoprotection was achieved with triple therapy (PIRF + LOS + MMF), which significantly decreased albuminuria, glomerulosclerosis, collapsed G and interstitial fibrosis, and was associated with a normal weight gain.

These results confirmed that pirfenidone has anti-fibrotic effects. Pirfenidone associated with LOS and MMF induced the best renoprotective effect in this model, and may be considered a promising alternative for the treatment of chronic progressive nephropathy.

W-PO20061

EXPRESISON OF CTGF MRNA AND TGF-BETA 1 MRNA IN TUBULOINTERSTITIAL AREA ASSOCIATED WITH URINARY PROTEIN IN IGA NEPHROPATHY

SACHIKO TAKAHASHI[1], TAKAYUKI FUJITA[1], YUKI WADA[1], KEN ITO[1], YOSHINOBU FUKE[1], ATSUSHI SATOMURA[1], KOICHI MATSUMOTO[1]

Division of Nephrology and Endocrinology Department of Internal medicine, Nihon University School of medicine[1]

Background: Tubulointerstitial fibrosis may become the prognostic indicator in IgA nephropathy (IgA-N). Many factors are known to be key mediators of tubulointerstitial fibrosis. Recently, connective tissue growth factor (CTGF) was observed to be strongly upregulated in human proliferative and fibrogenic diseases. In IgA-N, CTGF may play an important role in the development and progression of tubulointerstitial fibrosis. We examined the expression and localization of CTGF mRNA and TGF-beta 1 mRNA in the tubulointerstitial area using rapid in situ hybridization and its association with urinary protein.

Methods: Renal biopsy specimens were obtained from 10 patients with IgA-N. None of the patients had been treated with steroid or immunosuppressive drugs before the renal biopsy. We examined the expression of CTGF mRNA and TGF-beta 1 mRNA in renal tissue using a rapid in situ hybridization, and assessed the relationship between urinary proteins. The clinical parameters were examined at the time of renal biopsy.

Results: Our rapid in situ hybridization method demonstrated the presence of positive staining for CTGF mRNA and TGF-beta 1 mRNA in tubular epithelial cells in all renal tissue specimens. The positive cells for CTGF mRNA and TGF-beta 1 mRNA were higher in patients with heavy proteinuria than the patients with mild proteinuria. Very faint positive signals were observed in the fibrotic area.

Conclusion: These findings suggest that expression of CTGF mRNA and TGF-beta 1 mRNA were upregulated in the tubulointerstitial area and these expressions are associated with urinary protein in IgA-N.

W-PO20062

LOCALISATION OF PROTEOLYTIC ACTIVITY, MATRIX METALLOPROTEINASES (MMP) AND THEIR INHIBITORS (TIMPS) IN EXPERIMENTAL KIDNEY SCARRING

AIMUN K. H. AHMED[1], E. KHEDR[1], A. M. EL NAHAS[1], T. S. JOHNSON[1]

Sheffield Kidney Institute (University of Sheffield), Northern General Hospital, Sheffield, United Kingdom[1]

Background: Reduced MMP activity contributes to the ECM accumulation characterising kidney scarring. Homogenate studies show reduced proteolysis occurs predominantly due to overexpression of TIMPs. However homogenate studies fail to take into account *in-vivo* compartmentalisation and separation and may therefore be artefactual. To address this we have localised both ECM proteolysis and specific components of the MMP and TIMP families within kidney sections.

Methods: Wistar Rats were subjected to $5/6^{th}$ subtotal nephrectomy (SNx) to induce kidney scarring. Groups of 4–6 rats had the remnant kidney recovered between 7 & 120 days following surgery. Overall changes in ECM proteolysis in homogenates was measured. Localisation of proteolysis was performed using *in-situ* Zymography. Immunhistochemistry was used to localize MMPs 1,2 3 &9 and TIMPs 2&3 within the scarring kidney and TIMP1 by western blotting.

Results: Gelatinases activity was reduced from day 7 post SNx onwards by 49% ($p < 0.01$), collagenases activity decreased from day 60 (79% reduction, $p < 0.05$) onwards.

In-situ Zymography (Collagen IV substrate) showed proteolytic activity in normal kidney to be predominantly cytoplasmic within the tubules. This was decreased by 59 and 81% at 90 and 120 days post SNx respectively. In contrast, glomerular proteolytic activity increased in the SNx, 27 fold by 120 days.

Immunohistochemistry for MMPs 1, 2, 3 & 9 and TIMPs 2&3 confirmed the *in-situ* zymography location within the tubules. By day 120, MMP1 immunostaining increased from 0.1 to 7%, and MMP2 by from 0.27 to 9.36%. In contrast MMP3 decreased by 55% and TIMP2 increased by 31%, Western blot analysis of TIMP1 showed increase by 320.

Conclusion: ECM proteolytic activity is reduced in homogenates from scarred kidneys with changes in MMPs and TIMPs occurring in the same cellular compartment. However the vast bulk of this proteolytic potential is cytoplasmic and that contradicts to the interstitial location of ECM accumulation in scarring.

W-PO20063

ANTI-FIBROTIC VERSUS PRO-FIBROTIC: DUAL POTENTIAL OF ALL-TRANS RETINOIC ACID IN MESANGIAL CELLS AND RENAL FIBROBLASTS

QIHE XU[1], AYDA YEGANEH[1], YING HONG[2], XIONGZHONG RUAN[1], JAVIER LUCIO-CAZANA[3], JILL T NORMAN[1]

Department of Medicine, Royal Free and University College Medical School, London, United Kingdom; Department of Nephrology, General Hospital of Chinese PLA, Beijing, China[1]*, Wolfson Institute for Biomedical Research, Royal Free and University College Medical School, London, United Kingdom*[2]*, Departamento de Fisiologia, Facultad de Medicina, Universidad de Alcala, Alcala de Henares, Madrid, Spain*[3]

All-*trans* retinoic acid (tRA) suppresses both inflammatory and fibrotic changes in experimental nephritides, however, whether tRA has inflammation-independent anti-fibrotic effects remains controversial. We hypothesise that tRA may play an anti-fibrotic role by targeting the major extracellular matrix (ECM)-producing cells, renal mesangial cells and fibroblasts. To test this hypothesis, we examined the effect of tRA (1–5 mM) on the expression of fibrogenic genes in primary cultures of human mesangial cells (HMC) and a rat kidney fibroblast cell line (NRK-49F).

Results: (1) tRA suppressed transforming growth factor-b1 (TGF-b1)-induced fibronectin, collagen III and connective tissue growth factor (CTGF) mRNA expression in both HMC and NRK-49F cells. (2) tRA suppressed TGF-b1-induced collagen I mRNA expression in HMC but not in NRK-49F cells. (3) In HMC, 10 mU/ml glucose oxidase induced expression of fibronectin and collagens I, III and IV at the mRNA level, which was blocked by catalase, a H_2O_2 scavenger, and also by tRA. In contrast, tRA slightly, but reproducibly, enhanced

TGF-b1-induced collagen IV and TGF-b1 mRNAs. (4) In HMC, lower concentrations of H_2O_2 (glucose oxidase, 1–5 mU/ml) did not induce expression of fibrogenic genes, however, both tRA and catalase enhanced the expression of fibronectin and collagen mRNAs in the presence or absence of 1–5 mU/ml glucose oxidase. (5) In HMC, hypoxia (1% O_2), high glucose (30 mM), angiotensin II (1–5 mM), endothelin-1 (5 nM) and CTGF (100 ng/ml) had small and variable effects on ECM mRNA expression but both the basal and inducible expression of fibronectin, collagen III and CTGF mRNAs was suppressed by tRA.
Conclusion: tRA has both anti-fibrotic and pro-fibrotic potential in a cell type- and redox-dependent manner; it exerts its effects by actions on TGFb1-dependent and -independent changes. Further investigations are required to evaluate the efficacy of this emerging agent in the treatment and prevention of renal fibrosis.

W-PO20064

GLYCATION OF ALBUMIN MARKEDLY INCREASES ITS NEPHROTOXICITY

MARIE-LUISE PAMELA GROSS[1], ANGELIKA BIERHAUS[2], MERYEM YÜKSELEM[1], KATERINA NEUMANN[1], IRINA BERGER[1], NADJA KOLEGANOVA[1], EBERHARD RITZ[2]
Institute of Pathology, University of Heidelberg[1], Dept. of Internal medicine, University of Heidelberg[2]

Increasing evidence has been adduced that proteins in tubular fluid are nephrotoxic. After uptake into proximal tubular epithelial cells, an inflammatory phenotype is induced, agonists, cytokines and chemokines are secreted in a preferentially abluminal direction and interstitial fibrosis is provoked. In vivo it is difficult to study protein loading of tubular epithelial cells in isolation, i.e. without glomerular damage, renal hemodynamic changes etc. And there is an ongoing debate whether albumin per se is nephrotoxic.
Recently an amphibian model (Axolotl) has been described which took advantage of the unique anatomy of the kidney which is composed of closed as well as 'open' nephrons. The latter are connected with the peritoneal cavity by an orificium so that injection of protein i.p. selectively causes protein storage and peritubular fibrosis.
Endotoxin free preparations of glycated albumin and other forms of metabolized albumin were injected i.p. into Axolotl and the kidneys were obtained after 10 days and studied by light microscopy and immunehistochemistry (TGFβ, collagen I and IV, PDGF).
Glycated albumin was strikingly more nephrotoxic as indicated by protein storage in tubular epithelial cells and peritubular fibrosis compared to unmodified albumin. Lipid oxidation and carbamylation were only moderately more nephrotoxic as indicated by higher scores.
The data indicate that glycation dramatically increases the nephrotoxicity of albumin.

Table: *Renal damage score with different albumin preparations [Score 0–4]*

	Protein storage	peritubular fibrosis	TGF-β expression of tubules
NaCl	1.23 ± 0.5	0.4 ± 0.05	0.45 ± 0.1
Glycated albumin	3.7 ± 0.4*	3.3 ± 0.6*	0.62 ± 0.1
Delipdated albumin	2.4 ± 0.5*	2.45 ± 0.7*	1.0 ± 0.4*
Albumin lipid oxidation	2.6 ± 0.6*	2.3 ± 0.6*	1.16 ± 0.4*
Carbamylated albumin	2.8 ± 0.3*	2.67 ± 0.7*	1.49 ± 0.6*
Albumin	2.74 ± 0.4*	2.87 ± 0.7*	1.0 ± 0.4*
ANOVA	$p < 0.05$	$p < 0.05$	$p < 0.05$

* $p < 0.05$ vs. NaCl injection.

W-PO20065

URINARY EXCRETION OF THE COLLAGEN TYPE IV AS A PROGNOSTIC MARKER IN PATIENTS WITH PRIMARY GLOMERULONEPHRITIS

ILONA IDASIAK-PIECHOCKA[1], ANDRZEJ OKO[2], ELZBIETA PAWLICZAK[2], KRZYSZTOF PAWLACZYK[2], STANISLAW CZEKALSKI[2]
Department of Nephrology, University School of Medical Sciences, Poland[1]

The aim of this study was an evaluation of urinary excretion of the collagen type IV in patients with newly diagnosed primary glomerulonephritis (GN).
Forty three untreated patients (35 M, 8 F, and age 37.2 ± 10.5) with biopsy proven GN participated in the study. In the kidney biopsy specimens, the per cent of the sclerotized glomeruli and the intensity of interstitial fibrosis were assessed. In the first freshly voided morning urine sample the concentration of collagen type IV (ELISA method) and creatinine were measured and urinary excretion of the collagen type IV (coll.t.IV) was expressed as coll.t.IV/creatinine ratio. The results obtained in healthy subjects matched for sex and age, served as control (4.7 ± 2.1 ng/gCr).
Significantly elevated urinary coll t.IV excretion (20.0 ± 17.1 ng/gCr) was found in 19 patients with more than 10% of sclerotized glomeruli and pronounced interstitial fibrosis compared to 24 patients with less marked morphological changes (7.9 ± 5.7 ng/gCr; $p < 0.01$). Increased urinary coll t.IV excretion was also found in 12 patients with elevated serum creatinine level (Scr 1.6 ± 0.2 mg/dl) when compared with 31 patients with normal Scr (coll.t.IV 25.1 ± 19.3 ng/gCr vs 8.6 ± 6.6 ng/gCr; $p < 0.0005$). A correlation between protein and coll.t.IV excretion was found ($p < 0.03$), but there was no significant difference in coll.t.IV excretion between patients with and without nephritic syndrome, or with and without hypertension. Immunosuppresive therapy was introduced in 30 patients and in 13 patients conservative treatment was recommended. The remission or marked reduction of proteinuria with stabilization of kidney function was reached in 29 patients with urinary coll.t.IV excretion of 5.2 ± 3.0 ng/gCr before treatment. Fourteen patients did not respond to treatment and their urinary coll.t.IV excretion before therapy was markedly higher (29.8 ± 18.4 ng/gCr; $p < 0.000001$).
These results suggest that the determination of urinary coll.t.IV excretion before initiation of treatment in patients with GN may serve as the indirect indicator of the severity of kidney injury and as prognostic marker of the response to therapy.

W-PO20066

THE EFFECTS OF ROSIGLITAZONE ON THE EXPERIMENTAL RENAL TUBULOINTERSTITIAL FIBROSIS OF RATS

HUI GAO[1], ZI LI[1], WEI QIN[1], BAIHAI SU[1], JING LI[1], JUNMING FAN[1]
Division of Nephrology, Department of Medicine, West China Hospital of Sichuan University, Chengdu, Sichuan, China 610041[1]

To illuminate whether rosiglitazone exerted a role to block TEMT in vivo and its anti-fibrotic mechanism.
Method: Sixty male wistar rats were randomly assigned into unilateral ureteral obstruction (UUO), rosiglitazone large dose (PLD), rosiglitazone small dose (PSD), sham-operated (SOR) and ACEI groups. From the first day of initial UUO, PLD, PSD and ACEI groups were administrated intragastrically with rosiglitazone 8 mg/kg, 4 mg/kg and fosinopril 10 mg/kg daily. Identical voluminal normal saline was administrated to UUO and SOR groups. At 7, 14, 21, 28 days after UUO, 3 randomly selected rats of each group were sacrificed. Serum creatinine, blood cell differential counts, blood-fasting sugar (BFS), glutamate-pyruvate transaminase (GLT), 24-hour urine protein levels were measured. In HE and masson staining pathological slides, tubulointerstitial damage degree was scored. Expression of CTGF, BMP-7, α-SMA, CD68, fibronection (FN), TIMP1, MMP3 were detected by immunohistochemitry. Localization and expression of CTGF and BMP-7 protein were detected by hybridization in situ.
Results: Serum creatinine was significantly higher in UUO group than other groups at day 7, 14, 21, 28 ($p < 0.05$), but not in 24-hour urine protein, BFS and GLT. Neutrophil count, categorization and expression of CTGF, TIMP1, colIII and FN were increased conspicuously in UUO group ($p < 0.01$). α-SMA-positive interstitial cells number increased at day 14–21 of UUO. Renal BMP-7 mRNA and protein levels decreased progressively during the progression of obstructive nephropathy. Compared with the UUO group, levels of BMP-7 mRNA and protein increased significantly in PLD, PSD and ACEI groups. Levels of BFS and GLT in PLD and PSD were not increased markedly. Expression of CTGF, α-SMA, FN, ATIMP1, AMMP-3 in PLD and PSD groups were parallel to AECI group.
Conclusion: Rosiglitazone may suppress fibrosis, via down-regulating CTGF and up-regulating BMP-7 expression in affected kidney. It can also extenuate renal inflammation and decrease accumulation of extracellular matrix.

W-PO20067

EFFECTS OF LIPO-PGE1 AND DEXAMETHASONE ON EXPERIMENTAL FOCAL SEGMENTAL GLOMERULOSCLEROSIS IN RATS

SHENG LI REN[1], CHANG YING XING[2], DONG HUI ZHENG[2], WEI CHUN HE[2], XIU FEN ZHAO[2], JUN QIAN[2]
Department of Nephrology, First Affiliated Hospital of Nanjing Medical University, Nanjing, and Department of Nephrology, the Affiliated People's Hospital of Jiangsu University, Zhen Jiang, China[1], First Affiliated Hospital of Nanjing Medical University[2]

To study the effects of Lipo-PGE1 and dexamethasone (Dex) on experimental focal segmental glomerulosclerosis in rats. Forty SD rats were randomly divided into following groups: experimental focal segmental glomerulosclerosis model group (n = 8), Lipo-PGE1 treatment group (n = 8), Dex treatment group (n = 8), Lipo-PGE1 and Dex combination treatment group (n = 8), normal control group (n = 8). Rats in normal control group were subjected to sham operation and injected with normal saline after one week through the tail vein. Rats in other groups were uninephrectomized and injected with adriamycin (5 mg/kg) after one week through the tail vein. The dose of Lipo-PGE1 was 200 ug/kg/d. The dose of Dex was 200 ug/kg/d. Urinary protein excretion, serum total protein and albumin, serum creatinine (Scr), blood urea nitrogen (BUN) were measured at 2nd, 6th and 10th week after operation. Renal pathology was evaluated at the 10th week. Immunohistochemistry was used to examine the expression of collagen I, III, laminin (LN), TGF-β1, CTGF in the glomerular tissue. Lipo-PGE1 not only reduced urinary protein and BUN, but also significantly reduced glomerular mesangial proliferation and glomerular sclerosis (P 0.05). Immunohistochemistry staining indicated that increased collagen I, III, LN, TGF-β1, CTGF expression in model control group compared with treatment groups (P 0.01). At the same time, increased collagen I, III, LN, TGF-β1, CTGF expression in Dex treatment groups compared to model control group ($P < 0.05$). There was no prominent difference between Dex treatment groups and combination treatment group.

Conclusions: Lipo-PGE1 has a renoprotective effect on experimental glomerulosclerosis in rats, but Lipo-PGE1 can not reverse the injure causing by Dex. Lipo-PGE1 may inhibit the progression of focal segmental glomerulosclerosis by reducing the deposition of extra cellular matrix, which may be related to the down regulating expression of CTGF and TGF-β1.

W-PO20068

SEXUAL DIMORPHISM IN MESANGIAL CELL PROFIBROTIC RESPONSES: THE EFFECTS OF SEX HORMONES

EDDIE K. C. TAN[1], IZA PAWLUCZYK[1], KPG HARRIS[1]
John Walls Renal Unit, Leicester General Hospital[1]

Chronic Renal Failure progresses more rapidly in males than females. This may be due to positive effects of oestrogen or negative effects of testosterone. This study examined sex differences and the effects of sex hormones on profibrotic responses in mesangial cells.

Experiments were performed on rat mesangial cells (rmc) cultured from age-matched male and female Wistar rat kidneys.

Male rmc expressed higher basal fibronectin levels compared to females (164.6 ± 21.8 vs. 84.3 ± 9.2 mcg/mg, p = 0.001). To mimic rmc injury, cells were stimulated with macrophage conditioned medium (MCM). Fibronectin levels increased to 303 ± 31.6 mcg/mg cell protein in males and 170 ± 31 mcg/mg cell protein in females, p = 0.006. However, the fold increase was not significantly different between the sexes (3.89 ± 1.22 and 3.13 ± 0.87 in male and female rmc, respectively).

TNF-α and IL-1β are pleiotropic cytokines involved in inflammation and scarring. Basal TNF-α and IL-1β were higher in male than female rmc (165 ± 53 vs. 82.5 ± 7.3 and 14.3 ± 5.3 vs.10 ± 1.4 pg/mg cell protein, respectively). MCM stimulation further elevated TNF-α and IL-1β levels in male and female rmc, but the sex difference was maintained (412 ± 96 vs. 281 ± 8.5 and 25.9 ± 5.6 vs. 17.6 ± 0.8 pg/mg cell protein, respectively).

Control and MCM-stimulated male and female rmc were treated with 10^{-10}–10^{-7} M 17-βestradiol or testosterone. 10^{-8} M 17-βestradiol downregulated basal fibronectin levels in female rmc (24.1 ± 7%, p = 0.027) but had no effect on male rmc. Testosterone had no effect on basal fibronectin levels in either sex. 10^{-8} M testosterone increased fibronectin levels in MCM-stimulated male and female rmc (23 ± 6.9%, p = 0.029 and 32 ± 8.5%, p = 0.02). Testosterone upregulated TNF-α (23.2 ± 8.4% p = 0.026) and IL-1β (6 ± 0.6%, p = 0.0004) in female but not male MCM stimulated rmc.

In conclusion, male rmc exhibit a more profibrotic phenotype than female rmc. Testosterone appears to exacerbate this phenotype in the context of mesangial cell injury. 17-βestradiol appears less important. These observations may explain the accelerated progression of kidney disease observed in males.

W-PO20069

FIBROBLAST-SPECIFIC PROTEIN 1 IS A SPECIFIC PROGNOSTIC MARKER FOR LONG-TERM RENAL SURVIVAL IN PATIENTS WITH IGA NEPHROPATHY

YOSHIHARU NISHITANI[1], MASAYUKI IWANO[2], YUKINARI YAMAGUCHI[2], KOUJI HARADA[2], KIMIHIKO NAKATANI[2], YASUHIRO AKAI[2], TOSHIHIKO NISHINO[2], MASAO KANAUCHI[2], YOSHIHIKO SAITO[2], HIDEO SHIIKI[3], ERIC G NEILSON[4]
Haibara general hospital[1], First Department of Internal Medicine / Nara Medical University / Japan[2], Internal Medicine / Haibara General Hospital / Japan[3], Departments of Medicine and Cell and Developmental Biology / Vanderbilt University School of Medicine / USA[4]

Background: There has been no definitive evidence fibroblasts are involved in the initiation and progression of the renal interstitial fibrosis in human glomerulonephritis. With the availability of a new specific marker for fibroblasts, we determined the presence of fibroblasts in kidneys with IgA nephropathy (IgAN) and correlated their numbers with various parameters. We also prospectively analyzed to determine if the number of fibroblasts in the renal interstitium correlates with prognosis.

Methods: Cells positive for fibroblast-specific protein 1 (FSP1) were localized in renal biopsy specimens immunohistochemically using a polyclonal anti-FSP1 antibody. In 142 patients with normal serum creatinine concentrations, the relationship between the number of FSP1+ fibroblasts and renal survival was evaluated using Cox proportional hazards.

Results: Fibroblasts identified by their expression of FSP1 accumulated in areas showing severe interstitial fibrosis. Some tubular epithelial cells undergoing epithelial-meschymal transition (EMT) in fibrotic areas also expressed FSP1. The number of FSP1+ fibroblasts correlated directly with serum creatinine (r = 0.73, $p < 0.0001$), though multivariate analysis indicated the clinical factors influencing renal survival were urinary protein excretion (>=3.0 g/day, relative risk = 6.3, p = 0.03), hypertension (23.3, p = 0.0057), and >=20 FSP1+ fibroblasts per HPF (19.9, p = 0.015). Staining for FSP1+ fibroblasts was largely non-overlapping with aSMA+ interstitial cells.

Conclusion: The target protein FSP1 identified human fibroblasts and tubular epithelium undergoing EMT, and distinguished them from the diaspora of aSMA+ vascular smooth muscle cells. FSP1+ fibroblasts are critically related to the progression of IgAN; consequently, staining FSP1 in renal biopsy specimens provides a valuable histological index of progression.

W-PO20070

EXPRESSION OF APOLIPOPROTEIN H IN CHILDHOOD PRIMARY NEPHROTIC SYNDROME

RUI FU[1], HONG XU[2], LIANG CHEN[3]
Division Of Nephrology, Children's Hospital Of Fudan University, China[1], Department Of Pathology, Children's Hospital Of University, China[2]

Objectives: To study the expression of apolipoprotein H (ApoH) in childhood primary nephrotic syndrome (PNS).

Methods: Immunohistochemistry staining and real-time quantitative polymerase chain reaction (RT-PCR) were used to study the expression of ApoH in the renal tissues of 78 patients with PNS, 14 normal renal tissues as controlsl. Serum albumin, serum lipid, proteinuria and retinol binding protein (RBP) in the urine were tested before renal biopsy.

Results: (1) There were positive expression of ApoH protein and ApoH mRNA in the renal tissues of PNS patients and normal controls, mainly in the proximal tubules. The expression of ApoH mRNA was positively correlated with the protein expression ($r = 0.264$, $p < 0.05$). (2) The level of ApoH mRNA and protein expression in renal tissue of MCNS and MN decreased slightly (4.95 ± 0.40, 4.73 ± 0.60, and 12.06 ± 2.04, 12.35 ± 0.61, respectively), no statistical significantion when comparing with that of the normal controls. The expression in MsPGN and FSGS decreased significantly (3.30 ± 1.28, 2.82 ± 0.36, and 10.13 ± 3.09, 10.12 ± 1.02, respectively), when comparing with that of MCNS, MN and the controls ($p < 0.001$). (3) There were also decreased expression of ApoH mRNA in steroid resistant group comparing to sreroid sensitive group (4.27 ± 0.30 vs 4.97 ± 0.99, $p < 0.05$). And lower expression in frequent relapse group

than in infrequent relapse group (4.15 ± 0.72 vs 5.03 ± 0.32, $p < 0.05$). (4) The lower expression of ApoH were shown in patients with higher degree of tubules lessons, higher degree of interstitial damage ($p < 0.01$, respectively) and those with higher level of urine RBP ($r = -0.697, -0.764$, $p < 0.01$).
Conclusions: ApoH could be expressed in the kidney. It might play some roles in preventing interstitial fibrosis in nephrotic syndrome in children.

W-PO20071

INHIBITION OF CYTOKINE-INDUCED MMP-9 EXPRESSION IN RAT RENAL MESANGIAL CELLS BY CYCLOSPORIN A AND RAPAMYCIN

JOSEF PFEILSCHIFTER[1], W. EBERHARDT[2], V. ULBRICHT[3], E.S. AKOOL[4], H. RAU[3], C. KUROWSKI[4], [2], R. MÜLLER[3]
University Hospital Frankfurt[1], pharmazentrum frankfurt/ZAFES; Klinikum der Johann Wolfgang Goethe-Universität Frankfurt am Main[2], 60590 Frankfurt[3], Germany. pharmazentrum frankfurt/ZAFES; Klinikum der Johann Wolfgang Goethe-Universität Frankfurt am Main[4]

The expression of various inflammatory genes such as inducible nitric oxide synthase (iNOS) or matrix metalloproteinase-9 (MMP-9) is triggered in glomerular mesangial cells (MC) by exposure to the inflammatory cytokine interleukin 1b (IL-1b). We investigated the effects of cyclosporin A (CsA) a potent immunosuppressive drug on the cytokine-induced MMP-9 expression. Long-term treatment with CsA can cause severe renal side-effects clinically manifested as CsA-induced nephropathy. We found that the addition of CsA dose-dependently suppressed the IL-1b-induced MMP-9 activity in conditioned media of MC. Concomitantly there was a reduction in the MMP-9 mRNA steady-state level as demonstrated by Northern blot analysis.
By testing further immunosuppressive drugs we found that FK 506 (tacrolimus) has no inhibitory effects on MMP-9 expression, whereas rapamycin (sirolimus), similarly to CsA, inhibited the IL-1b-induced MMP-9 expression. Searching for possible transcriptional effects we found that CsA inhibited cytokine-induced luciferase activity of a 1.8 kb fragment of the rat MMP-9 promoter. By electrophoretic mobility shift assay (EMSA) we observed an inhibition of the cytokine-induced DNA-binding of nuclear factor kappa B (NF-kB) to its MMP-9-specific binding-site. The inhibition in DNA binding was paralleled by a reduction of the content of nuclear p65 subunit of NF-kB, whereas the nuclear content of c-Jun a member of the AP-1 transcription factor remained unchanged. Our data indicate that CsA and rapamycin by reducing the expression of matrix metabolizing proteases such as MMP-9 may cause accumulation of extracellular matrix (ECM) and thereby facilitate fibrotic processes in the kidney.

W-PO20072

ANGIOTENSIN II RECEPTOR BLOCKERS ATTENUATED EXPRESSION OF FIBROGENIC CYTOKINES ON CULTURED RAT RENAL INTERSTITIAL CELLS

AKIRA YAMAMI[1], TAKESHI FUKAI[1], HIROKO NUMANO[1], KEIKO YAMADA[1], SEN-ICHIRO OOKI[1], TOMOHIRO MORIO[1], TAKASHI ARAI[1], SHOJI KUWATA[1]
Teikyo University School of Medicine Ichihara Hospital[1]

Objective: Fibrogenic cytokines such as TGF-beta1, PDGF-A and TIMP-1 have been reptored to be involved in advocating renal interstitial fibrosis. Angiotensin II receptor blockers (ARBs) have been considered to reduce the progression of cardiac muscle fibrosis. We investigated the role of ARBs on suppression of fibrogenic cytokines in cultured rat renal interstitial cells.
Methods: Renal outermedullary interstitial cells (ROMIC) from SD rats were cultured in Dulbecco's modified medium. Candesartan, valsaltan and telmisaltan were added in cultured medium in ARB study. Gene expression of TGF-beta1, PDGF-A and TIMP-1 were measured using RT-PCR method. Thin slices of rat renal tissue were also provided for examining tissue distribution profile of cytokine production.
Results: ROMIC and renal tissue specimens expressed unequivocally TGF-beta1, PDGF-A and TIMP-1. Renal cortex expressed least among renal tissue slices. All ARBs reduced the gene expression by 8–23%.
Conclusions: Renal interstitial cells participate in the progression of renal interstitial fibrosis through releasing fibrogenic cytokines. ARBs decreased the production of fibrogenic cytokines and retrieved the progression of renal interstitial fibrosis.

W-PO20073

PROTEIN RESTRICTION AND AST-120 IMPROVE LIPOPROTEIN LIPASE AND VLDL RECEPTOR IN FOCAL GLOMERULOSCLEROSIS

TADASHI SATO[1], KAIHUI LIANG, NOSRATOLA D. VAZIRI[2]
Saga University[1], Nephrology and Hypertension University of California Irvine California USA[2]

Background: Imai rats exhibit spontaneous focal glomerulosclerosis (FGS) with progressive proteinuria and hyperlipidemia leading to renal insufficiency by age 34 weeks. Recently, we reported marked down-regulations of skeletal muscle and adipose tissue lipoprotein lipase (LPL) and very low-density lipoprotein (VLDL) receptor in male Imai rats at 32 weeks of age. Dietary protein restriction and oral adsorbent AST-120 (AST) have been shown to slow progression of renal disease and attenuate hyperlipidemia in the Imai rats. This study tested the hypothesis that amelioration of proteinuria by protein restriction or use of oral adsorbent AST-120 beginning at 10 weeks of age may improve renal disease and LPL and VLDL receptor deficiencies in Imai rats.
Methods: Ten-week-old male Imai rats were randomly assigned to those fed either a regular diet, low protein diet (LPD), or regular diet containing the adsorbent preparation, AST-120. Ten-week-old male Sprague-Dawley rats served as controls. The animals were observed for 24 weeks. Six rats were included in each group. All diets were prepared in powder form.
Results: The untreated 34-week-old Imai rats showed severe proteinuria, hypoalbuminemia, 50% reduction in creatinine clearance, hypercholesterolemia, hypertriglyceridemia, and elevated plasma VLDL concentration. This was associated with significant reductions in plasma post-heparin LPL activity, hepatic lipase activity, as well as adipose tissue and skeletal muscle immunodetectable LPL and VLDL receptor proteins. Protein restriction mitigated the decline in creatinine clearance, ameliorated proteinuria, hypoalbuminemia, hypertension, and hypercholesterolemia, lowered plasma VLDL, and improved plasma postheparin LPL activity, hepatic lipase activity, LPL, and VLDL receptor proteins in skeletal muscle and adipose tissue. Similar improvements were observed in all parameters with AST administration.
Conclusion: Moderate protein restriction and use of oral adsorbent can slow progression of renal disease and, thereby, ameliorate LPL, hepatic lipase, and VLDL receptor deficiencies and the associated hyperlipidemia in rats with spontaneous FGS.

W-PO20074

RELATIONSHIPS BETWEEN CIRCULATING MATRIX METALLOPROTEINASE-2 AND -9 VERSUS RENAL FUNCTIONS IN PATIENTS WITH CHRONIC KIDNEY DISEASES

HORNG-RONG CHANG[1], JONG-DA LIAN[2], SHUN-FA YANG[3], CHIU-CHU LIN[3], YIH-SHOU HSIEH[3], MEI-LAN LI[1]
Institude of Medicine, Chung Shan Medical University, Taichung[1], Division of Nephrology, Department of Internal Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan[2], Institute of Biochemistry and Biotechnology, Chung Shan Medical University, Taichung, Taiwan[3]

Background: It has been proven that extracellular matrix turnover is involved in the pathogenesis of renal fibrosis. Matrix metalloproteinase-2 and -9 (MMP-2 & -9) are the extracellular matrix degrading enzymes that are believed to play important roles in many diseases. But, the role of circulating levels of MMP-2 and -9 in the patients with chronic kidney diseases (CKDs) has not yet been clear.
Methods: Gelatin zymography was employed to measure MMP-2 and -9 activities in the plasma of 50 patients with CKDs and 50 control subjects.
Results: The serum creatinine (SCr: 6.92 ± 0.51 mg/dl, SD ± SEM) and the MMP-2 activity (16782 ± 695) were significantly higher ($p < 0.001$) and the MMP-9 activity (16332 ± 1580) was significantly lower ($p < 0.05$) in the patients with CKDs compared with those (SCr: 0.77 ± 0.027 mg/dl; MMP-2, 9640 ± 590; MMP-9, 22247 ± 1606) of the control subjects. In addition, the SCr correlates with the MMP-2 activity ($r = 0.352$; $p < 0.05$) and inversely correlate with the MMP-9 activity ($r = 0.405$, $p < 0.01$) in the patients with CKDs.
Conclusions: Our results suggested that MMP-2 and -9 might play important roles in the pathogenesis of chronic renal failure.

Pathogenesis of Progression – Vascular Biology and Haemodynamics

W-PO20075

EFFECT OF ACUTE RENAL FAILURE ON THE PROGRESSION OF PREEXISTENT CHRONIC RENAL FAILURE IN RATS

MIRIAN A. BOIM[1], AURILEIDE ALVES LEITÃO[1], AURILUCIA ALVES LEITÃO[1], CLEBER A. LEITE[1], NESTOR SCHOR[1]
Universidade Federal de São Paulo[1]

The progression of chronic renal failure (CRF) may be accelerated by association of acute renal failure (ARF). This study evaluated the effect of the association of ARF induced by gentamicin (G) to the preexistent CRF induced by 5/6 nephrectomy. G (40 mg/kg/day) was given during 10 days to normal or 30 days CRF rats. Renal function and kidney histology were evaluated immediately (G, n = 5 and CRF + G, n = 4) or 30 days after G treatment (G30, n = 5 and CRF + G30, n = 8). Results were compared to control rats (C, n = 4) or CRF untreated rats (CRF30, n = 3 and CRF60, n = 11). Additional animals received the anti-inflammatory agent alpha melanocyte stimulating hormone (MSH, 50 mg/kg/day during 10 days) together with G treatment. G group presented reduction in creatinine clearance (Clcreat) and increase in sodium excretion characterizing the ARF caused by G. These parameters normalized after 30 days indicating the reversible character of this pathology. Clcreat decreased in CRF60 but not in CRF30 group demonstrating the initial adaptive response and the late progression of CRF typical of this model. CRF60 animals also presented tubular hypertrophy and glomerular sclerosis. The G association with CRF did not modify the renal function compared to CRF untreated rats, indicating a relative protection of CRF animals to the toxic effects of G. In contrast, the long term analyzes showed acceleration in the progression of CRF, manifested by an impressive increase in the proteinuria (130%), glomerular and tubular lesions. MSH minimized the long term effects of CRF associated or not with G. These results suggest that the CRF apparently minimizes the toxic effect of G when analyzed in short term, however the long term analysis reveled a faster degeneration of renal function in CRF rats. Inflammatory components are probably involved.

W-PO20076

ROLE OF CD40-CD154 INTERACTION IN THE PATHOGENESIS OF VASCULAR CALCIFICATION IN PATIENTS WITH END-STAGE RENAL FAILURE

VALENTINA CAMPEAN[1], D. NEUREITER[1], B. NONNAST-DANIEL[2], C. GARLICHS[3], M.L. GROSS[4], K. AMANN[1]
Pathology, University of Erlangen-Nürnberg, Erlangen, Germany[1]*, Internal Medicine IV University of Erlangen-Nürnberg Germany*[2]*, Internal Medicine II University of Erlangen-Nürnberg Germany*[3]*, Pathology University of Heidelberg Germany*[4]

Background: Patients with chronic renal failure develop accelerated atherosclerosis with increased vessel and plaque calcification the pathogenesis of which is not completely known. Among other risk factors, chronic microinflammation as reflected by increased CRP levels is likely to be involved. Activation of cells and adhesion molecules in atherosclerosis is governed by CD40-CD154 interaction. Therefore, we investigate the expression and distribution of CD40 and CD154 in different stages of calcified and non-calcified atherosclerotic lesions of patients with chronic renal failure compared to non-renal control patients.
Material and Methods: Coronary arteries from 47 patients with and without chronic renal failure were classified from type II to type VIII according to the classification of Stary and were analysed for in-situ protein expression of CD40, CD154 and CRP using immunhistochemistry and a semiquantitative scoring system. In parallel, infiltrating cells were characterized and correlated to the different stages of atherosclerotic lesions.
Results: CD40 protein was overexpressed in media myocytes of atherosclerotic plaques of renal and non-renal patients. Inside the plaques, CD40 protein was detected on endothelial cells, T-lymphocytes, macrophages, fibroblasts, and smooth muscle cells. The expression of CD40 differs between the plaque subregions. CD154 was identified in areas heavily infiltrated by strongly positive macrophages. Furthermore, the expression of CD40 and CD154 proteins was markedly enhanced in calcified versus non-calcified lesions from patients with chronic failure compared to non-uremic controls.
Conclusion: The data indicate involvement of CD40-CD154 signaling in the pathogenesis of progressive atherosclerosis in patients with chronic renal failure.

W-PO20077

PROTEINURIA AND THE PERITUBULAR CAPILLARY CHANGE: POTENTIAL ROLE OF ALBUMIN EXCESSIVE UPTAKE MEDIATED BY CUBILIN

YANI HE[1], LIANG H. J.[1], YANG J. R.[1], LIN JING[1], LI X. L.[1]
Dept. of Nephrology, Daping Hospital of TMMU[1]

Background: Over-reabsorption of filtered protein has been demonstrated to play a critical role in tubular and interstitial cell injury, leading to progressively interstitial fibrosis. Although peritubular capillary, the major component of renal tubulointersitium, is a 'close neighbour' of renal tubules and the loss of peritubular capillary was considered to be contributive to tubulointerstitial fibrosis, it has been not clear whether excessive uptake of filtered protein by renal tubular cells is responsible for the morphologic changes in peritubular capillary during the progression of nephropathy. In this study, we observed the renal peritubular capillary changes, albumin deposition and the expression of its endocytosic receptor, cubilin in the patients with nephrotic syndrome.
Methods: Thirteen patients with nephrotic syndrome and six patients with occulting hematuria were collected into our study. Albumin deposition, the expression of cubilin and the marker of endothelial cells (CD34), were detected by immunohistochemistry. Proliferating endothelial cells were immunohistochemically identified by double-labeling with anti-TM and anti-proliferating cell nuclear antigen (Ki67) monoclonal Ab.
Results: Comparing with the control group, the patients with nephrotic syndrome presented a larger amount of albumin deposition and stronger expression of cubilin in renal proximal tubular cells. The dense of peritubular capillary, numbers of Ki 67- and TM-positive proliferative endothelial cells also showed significant decreases in the proteinuric patients. The statistical result indicated there is a negative correlation between the albumin deposition in the renal proximal tubular cells and the dense of peritubular capillary (CD34, $\gamma = 0.68$, $P < 0.05$).
Conclusion: Excessive uptake of albumin in renal tubular cells may have close relationship with the changes of peritubular capillary in renal tissue. Cubilin, the endocytosic receptor of albumin may play an important role in this process.

W-PO20078

RENAL HAEMODYNAMICS IN ENOS(–/–) MICE

ZOLTAN H. ENDRE[1], MAJID RAHGOZAR[2], GLENDA GOBE[2]
Christchurch School of Medicine[1]*, University of Queensland*[2]

Renal haemodynamics are governed by autoregulation. Facilitation of autoregulation by angiotensin II (Ang II) is modulated by nitric oxide (NO). To investigate the role of selective NO synthase (NOS) isoforms, we compared renal haemodynamics in isolated perfused kidneys (IPMK) from eNOS(–/–) and wild type (WT) mice. GFR and fractional sodium excretion (FENa) in IPMK remained comparable with in vivo values in both WT and eNOS(–/–) kidneys after 90 min perfusion (GFR 399 ± 9 and 379 ± 7 µl.min-1gkw-1; FENa 1.5 ± 0.7 and 1.6 ± 0.6% respectively). As expected in these hypertensive animals, renal vascular resistance (RVR) was higher in eNOS(–/–) IPMK (3.7 ± 0.2 mmHg.min/ml) than WT (2.4 ± 0.1 mmHg.min/ml, p 0.05). The autoregulatory threshold was increased in eNOS(–/–) compared to WT IPMK (100–150 vs 60–110 mmHg, p 0.01). Autoregulation was partial in both eNOS(–/–) and WT kidneys (autoregulatory index (ARI) respectively 0.62 ± 0.1 and 0.65 ± 0.1, ns) and facilitated in both by infusion of 100 pM Ang II; facilitation was similarly inhibited by 50 µM methacholine. Autoregulation was facilitated by non-selective NOS inhibition with 50 µM L-NAME, decreasing ARI to 0.35 ± 0.1 and 0.46 ± 0.1 in WT and eNOS(–/–) respectively, p 0.05). Ang II produced no additional facilitation of autoregulation in the presence of L-NAME in either group. Facilitation by L-NAME was antagonised by 50 µM methacholine only in eNOS(–/–). Selective nNOS inhibition with 10 µM 7NI in eNOS(–/–) IPMK enhanced facilitation of autoregulation by Ang II, decreasing ARI from 0.44 ± 0.12 to 0.21 ± 0.05, p 0.05 but did not facilitate WT. With 7NI, AngII facilitation was also inhibited by 50 µM methacholine. We have previously shown that Ang II facilitates autoregulation by oxidatively decreasing NO. The results in eNOS(–/–) suggest that NO from nNOS in macula densa is a critical modulator of autoregulation and that this is masked in WT kidneys, probably by counter-regulatory NO generation from eNOS stimulated by Ang II.

W-PO20079

THE INFLAMMATION, BLOOD PRESSURE AND CHANGES IN ALPHA-1 ADRENERGIC RECEPTORS REACTIVITY OF ARTERIAL SMOOTH MUSCLE CELLS IN EXPERIMENTAL RENAL FAILURE

ANDRZEJ BRYMORA[1], MARIUSZ FLISIŃSKI[1], GRZEGORZ GRZEŚK[2], LESZEK SZADUJKIS-SZADURSKI[2], JACEK MANITIUS[1]
Dept. of Nephrology, Hypertension and Internal Diseases, Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz, Poland[1], Dept. of Pharmacology and Therapeutics Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz, Poland[2]

Introduction: The inflammation is a well-documented factor influencing the development of cardiovascular complications in chronic renal failure (CRF). The alpha-1 adrenergic receptors play a key role in the cardiovascular hemostasis. The aim of this study was to examine inflammatory status defined by level of serum haptoglobin and arterial response to phenylephrine in a different stages of renal failure.

Material and Methods: Rats (290–380 g) were divided into four groups: I (control) – shame-operated (n = 11), II – 1/2 nephrectomy (n = 7), III – 3/4 nephrectomy (n = 12), IV – 5/6 nephrectomy (n = 13). After 4 weeks blood pressure (BP) in carotid artery was measured and tail artery was excised. The blood was collected on BUN, creatinine, haptoglobin. Experiments were performed on isolated and perfused rat tail artery. We compared the smooth muscle contractility after stimulation of alpha-adrenoreceptor with phenylephrine (PHE) in groups.

Results: Blood pressure and labolatory tests are expressed on mean ± SD.

W-PO20080

IMPACT OF EXOGENOUS CHOLESTEROL ON CHOLESTEROLOGENESIS ACTIVITY IN EXPERIMENTAL CHRONIC RENAL FAILUIRE

MICHAL CHMIELEWSKI[1], BOLESLAW RUTKOWSKI[1], EWA KOSSOWSKA[2], JULIAN SWIERCZYNSKI[2], WOJCIECH BOGUSLAWSKI[3]
Department of Nephrology, Transplantology and Internal Medicine; Medical University of Gdańsk; Poland[1], Department of Biochemistry; Medical University of Gdańsk; Poland[2], Department of Medical Chemistry; Medical University of Gdańsk; Poland[3]

Hypercholesetrolemia in the course of chronic renal failure (CRF) is a significant risk factor for cardio-vascular complications. The main cause of this disturbance is increased cholesterol biosynthesis. It is activated even though the cholesterol content in plasma and hepatocytes is increased, pointing to the hypothesis that the physiological feedback inhibition of cholesterol synthesis is disturbed. The aim of the present study was to evaluate the impact of exogenous cholesterol on the activity of cholesterologenesis in experimental CRF. Wistar rats were used and experimental CRF was achieved by 5/6 nephrectomy model. Animals were included into one of four groups: controls fed a high (1%) cholesterol diet (C-H), controls fed a low cholesterol diet (C), CRF rats on a high cholesterol diet (CRF-H) and CRF animals fed a low cholesterol diet (CRF). Plasma cholesterol level in CRF rats was 129 ± 28 mg%, compared to 49 ± 5 mg% in control group ($p < 0.001$). Cholesterol concentration in livers of CRF rats (2.3 ± 0.3 mg%) was significantly higher than in control animals (1.9 ± 0.3 mg%) ($p < 0.02$). Activity of cholesterol biosynthesis, determined by incorporation of tritated water given *i.p.* into liver cholesterol of CRF animals was 59% higher than in C group. It was diminished over 5-fold in C-H animals, as compared to C rats. Similar decrease in cholesterologenesis rate was observed between CRF-H and CRF groups. Changes in the activity of 3-hydroxy-3-methylglutarl-CoA (HMG-CoA), the rate limiting enzyme in cholesterol biosynthesis are presented in the table. The values are presented in pmol/min × mg of microsomal protein.

	CRF rats	Control rats
low cholesterol diet	1167 ± 260	839 ± 246
high cholesterol diet	747 ± 275	591 ± 52
p	<0.01	<0.05

We conclude that the feedback inhibition of cholesterol biosynthesis by alimentary cholesterol is preserved in the course of experimental CRF.

W-PO20081

IMMUNOLOGICAL SCREENING OF THE AUTO-ANTIBODY RECOGNIZING COGAN SYNDROME RELATED PEPTIDE IN SYSTEMIC VASCULITIS

HITOSHI WATANABE[1], KEISUKE SUZUKI[1], YOSHINO MASABUMI[1], WATARU KITAGAWA[1], NAOTO MIURA[1], HARUTAKA YAMADA[1], MASAHITO SAKUMA[1], KAZUHIRO NISHIKAWA[1], HIROKAZU IMAI[1], ATSUSHI KOMATSUDA[1], HIDEKI WAKUI[2]
Department of Nephrology and Rheumatology Aichi Medical University[1], School of Medicine Third Department of Internal Medicine, Akita University School of Medicine[2]

Introduction: Cogan syndrome is a multisystem disease with chronic inflammatory change of unknown origin, characterized by progressive renal insufficiency sensorineural hearing loss, episcleritis, and vasculitis. In this pathogenesis, autoimmune processes were hypothesized because of the therapeutical effect in immunosuppressive therapy like prednisolon and cyclophosphamide. Recently, Claudio *et al* report the Cogan syndrome related autoantiboy which recognize the auto antigen to DEP-1/CD148, retrovirus major core antigen, 60 kD SSA/Ro protein and connexin 26 to be able to transfer the disease phenomenon in animals.

Method: The 20 cases of systemic vasculitis including Cogan syndrome, clinically diagnosed were analyzed the existence of auto-antibody using ELISA method. EIA plate coating chemically synthesized cogan syndrome related peptide was reacted with diluted serum, colorimetrically measured the density using peroxydase-labeled anti-human IgG. Optical density was assessed as the antibody-titer.

Result: 3 of 4 cases in Cogan syndrome have a high-titer auto-antibody, compared with healthy control. 7 of 16 cases in systemic vasculitis without Cogan syndrome have moderate titer. In one typical case, antibody titers were serially followed up for 2 months with clinical symptom. Under prednisolon and cyclophosphamide medication, antibody-titer was decreased according to have remission of urinaly protein excretion, sensoneural and vascular symptom.

Disscusion: Systemic vasculitis indicate the various symptom as the result of several organ obstruction due to micro vascular inflammatory change. Clinical specific marker is not many to diagnose the vasculitis. Cogan syndrome was mainly diagnosed according to characteristic clinical symptom including sensorineural hearing loss and episcleritis. Immunological detection of this auto antibody presents the helpful tool to diagnose the Cogan syndrome in systemic vasculitis. This autoantibody might be able to explain the several symptoms in vasculitis.

Summary: The autoantibody against cogan syndrome related peptide is detectable in serum of systemic vasculitis mainly including Cogan syndrome patients.

W-PO20082

ABNORMAL PRENYLATION OF RAS AND RHO IN HIGH GLUCOSE VASCULAR PATHOLOGY

AMANDA JANE MATHER[1], STELLA MCGINN[1], XIN CHEN[1], MICHAEL FIELD[1], CAROL POLLOCK[1]
Renal Research Group, Kolling Institute, Royal North Shore Hospital, University of Sydney, NSW[1]

Background: Isoprenoids induce post-translational modification of proteins such as the Ras and Rho families of GTPases. Once prenylated, these proteins affect kinases that regulate the cell cycle. Since exposure of endothelial cells to high glucose induces abnormalities in cell growth, our aim was to study the expression of prenylated GTPases in endothelial cells under such conditions. A role for these proteins in diabetic vascular disease may then be elucidated.

Methods: Human umbilical vein endothelial cells were grown for 72 hours in media containing 5 mM D-glucose (control), 25 mM D-glucose (high), or osmotic control (L-glucose). Isoprenylated and nonisoprenylated Ras and Rho were partitioned into detergent and aqueous phases and then measured, together with total protein, by Western blotting (n = 3). The prenylation status of the Ras isoform K-Ras was similarly assessed (n = 2). The influence of glucose on farnesyltransferase activity was assayed by measuring the transfer of labelled prenyl group from tritiated FPP into a recombinant Ras protein.

Results: HUVECs exposed to high glucose for 72 hours demonstrated no significant difference in expression of total Ras or RhoA compared to control. However, exposure of HUVECs to high glucose was associated with a 65% increase ($p < 0.05$) in the quantity of the prenylated form of Ras protein and a 95% increase in the prenylated form of RhoA ($p < 0.05$), independent of osmotic control. Preliminary data suggests that the prenylation status of K-Ras is also increased in high glucose conditions.

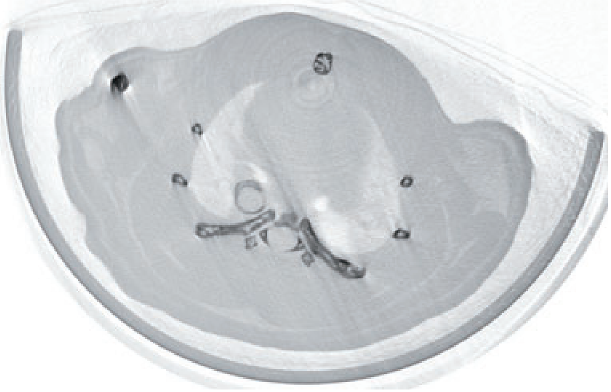
Conclusion: Exposure of endothelial cells to high glucose does not alter total Ras and Rho expression but does increase the expression of their prenylated forms. These data suggest that farnesyltransferase activity is increased under these conditions. Evaluation of the prenylation status of each of the Ras isoforms is currently under study to elicit their individual roles in the cell growth abnormalities that occur following high glucose exposure.

W-PO20083

DETECTION OF VASCULAR CALCIFICATIONS BY HIGH RESOLUTION X-RAY MICROTOMOGRAPHY IN LIVING RATS WITH CHRONIC RENAL FAILURE

VEERLE P. PERSY[1], ELLEN NEVEN[1], GEERT DAMS[1], MARC DE BROE[1], PATRICK D'HAESE[1], ANDREI POSTNOV[2], NORA DE CLERCK[2]
University of Antwerp – Nephrology[1], University of Antwerp – Microtomography[2]

Chronic renal failure (CRF) is associated with a 10 to 20-fold increase in cardiovascular risk. Vascular calcification (VC) is a prominent feature of cardiovascular disease in dialysis patients, and contributes to the excess mortality in this population. Adenine-induced CRF proved a suitable model to study uremia-related VC. This study explores micro-CT scanning as a tool to detect and follow-up VC in living uremic rats.
Thoracal micro-CT scans (Skyscan 1076, Belgium) were performed in rats under pentobarbital-anesthesia after 8 and 10 weeks of adenine-induced CRF. Scans (with Ti-filter) were isotropic, with a voxel size of 35 × 35 × 35 mm and 50 min scan time. Virtual cross sections were reconstructed by Feldkamp cone-beam algorithm. Tissue calcium content was measured by atomic absorption spectroscopy and VC was scored histologically on Von Kossa stained sections.
Micro-CT findings correlated well with tissue calcium content and histology. In 2/7 animals, micro-CT scanning clearly demonstrated extensive VC, visible as a dense delineation of the aortic wall (cfr. figure) over the whole scanned area. Aortic calcium content was 26.1 and 52.8 mg/g tissue, respectively and the tunica media showed circumferential Von Kossa positivity in all cross sections. One animal showed moderate calcification with focal Von Kossa positivity in 4/6 cross sections. In spite of the relatively low bulk calcium content (1.29 mg/g tissue), focal VC was observed in 19.6% of the micro-CT images. In 4 other animals no indications of VC were seen, neither with micro-CT scans nor histologically, and bulk calcium contents were not increased. 3D models of successive in vivo scans allowed quantitative follow-up of the calcification process.
In vivo micro-CT scanning is a sensitive method to detect calcifications in the thoracic aorta of CRF rats. In the future, this promising technique will allow to follow-up and quantify the development, and potential reversal during treatment, of vascular calcifications in living animals.



W-PO20084

IDENTIFICATION OF THE MINERAL PHASE OF TWO IN VIVO MODELS OF UREMIA RELATED VASCULAR CALCIFICATION

STEVEN C. VERBERCKMOES[1], VEERLE PERSY[1], GEERT J BEHETS[1], DOMINIK MÜLLER[1], DIETER HAFFNER[3], UWE QUERFELD[2], MARC E DE BROE[2], PATRICK C D[1]
Department of Nephrology-Hypertension University of Antwerp Belgium[1], Department of Pediatric Nephrology Charité University Berlin Germany[2]

Vascular calcification (VC) is a prominent feature of vascular disease in uremic patients. Mineral deposition in the medial part of the vessel in chronic renal failure (CRF) has been show to be a tightly regulated process in which vascular cells undergo a transdifferentiation to an osteoblastic phenotype.
Two models of uremia-related VC were used to identify the mineral phase deposited in the vascular wall. VC was induced in rats by either a two-week adenine treatment followed by a 10-week high (1.03%) phosphorus diet or 5/6th nephrectomy followed by 6 weeks of 0.25 ng/kg/d calcitriol treatment. Mineral identification was performed on 10?thick aortic sections by means of ?X-ray-diffraction using a 2 × 10?synchrotron beam spot size (beamline ID18F of the European Synchrotron Radiation Facility, Grenoble, France). Multi-element mappings for calcium and phosphorus were obtained using an X-ray-fluorescence detector. The mineral phase was identified by matching the integrated diffractograms to a mineral database. Sequential sections were stained with Von Kossa to show the presence of mineral deposits.
All investigated animals were Von Kossa positive, an observation which was confirmed by the positive signal for calcium and phosphorus in the X-ray-fluorescence mappings. In 1/4 animals with adenine-induced VC an amorphous precipitate was present with no identifiable mineral phase whilst in the other animals the mineral turned out to be hydroxyapatite. In the model of vitamin D-induced VC 3/9 animals showed an amorphous precipitate. One animal showed a mineral phase that consisted exclusively of hydroxyapatite while in the 5 remaining animals the occurrence of hydroxyapatite was accompanied with a whitlockite phase.
These data show that the mineral found in adenine-induced VC is hydroxyapatite, the mineral compound in bone. Whitlockite, as found in the vitamin-D-induced model of VC, is a magnesium-containing mineral and its presence may suggest a disturbed magnesium-uptake under influence of calcitriol-treatment.

W-PO20085

ALTERATIVE EXPRESSION OF ANGIOGENIC FACTORS DURING EARLY AND ADVANCED STAGES IN RENAL TISSUES OF EXPERIMENTAL CHRONIC RENAL ISCHEMIC RATS: FOCUS ON VEGF AND ITS RECEPTORS

JIAMING ZHU[1], XIAOQIANG DING[2], SHUAI JIA[2], CHENSHENG FU[2], XUNHUI XU[2]
Fudan University, Shanghai[1], Renal Division of Zhongshan Hospital, Fudan University, Shanghai, China[2]

Background: Chronic tissue hypoxia and impaired angiogenesis contribute to the progression of renal failure, however, the alterations of VEGF and its receptors are not completely understood in different stages of chronic renal ischemia.
Methods: Unilateral clamping of the left renal artery to induce chronic ischemia of the kidney was utilized in this study. At week 1 and 10 the left renal tissues were harvested. Hypoxyprobe^{TM}-1 was used to label the hypoxic extent. The renal expression of VEGF and its receptors (Flk, Flt) was dectected by immunohistochemistry and RT–PCR.
Results: The blood pressure and plasma creatinine of the experimental rats was elevated at week 6 and keep a high level in late stages. The left renal tissue with artery stenosis showed proximal tubuler epithelium vacuole and granulation degeneration with inflammatory cells infiltration in tubulerinterstitium in week 1 group. Hypoxyprobe^{TM}-1 labelling showed significant staining in out medulla and cortex, while only weak staining in outer medulla in the control rats. VEGF and its receptors Flk and Flt were markedly upregulated in one week ischemic renal cortex compared with the control, and the distribution was much similar with that of the Hypoxyprobe^{TM}-1 staining. But in advanced stage (week ten), the chronic ischemic renal tissue exhibited atrophic tubular epithelium with severe fibrosis. The Hypoxyprobe^{TM}-1 labelling showed limited staining only in medulla while negative staining in cortex, and VEGF and its receptors staining in cortex also weakened significantly compared with that of early stage (week one). Further investigation revealed a significant loss of peritubular capillaries in cortex area in advanced stages.

Conclusions: In early stage of renal ischemia, hypoxic condition induced VEGF and its receptors high expression in cortex and outer medulla, which would enhance the ability of angiogenesis to compensate oxygen supply. But persistent ischemia would finally decrease angiogenic factors expression, which accelerated ischemia extent of the kidney and attributed to renal failure in advanced stages.

W-PO20086

EFFECT OF DIHYDROPYRIDINE CALCIUM ANTAGONIST ON PROTEINURIA IN PATIENTS WITH SIGNIFICANT PROTEINURIA

CHEE-KHUN HO[1], TITUS W. LAU[1], EVAN J. C. LEE[1]
Department of Medicine, National University Hospital, Singapore[1]

Some studies have shown that dihydropyridine calcium antagonist (DHCCB) may worsen proteinuria. The intention of this study was to determine the effect of the use of DHCCB on proteinuria in patients with significant proteinuria (^{3}1 g/day).
This retrospective study included 135 consecutive patients who completed a minimum of 12 months follow-up in our center. All patients were on ACE inhibitors (ACEi) and/or ATII receptor antagonists (ATRA) unless contraindicated; DHCCB was added for blood pressure (BP) optimization. BP target was £130/80 mmHg. Patients were segregated by the presence of CCB use (patients on non-dihydropyridine CCB were excluded). Proteinuria and BP at baseline and 12-month were compared between the two groups.
Results: The study cohort had a mean age of 59 ± 11 years with 54% males and 75% diabetic nephropathy. Of the 135 patients, 59 (43.7%) received DHCCB. 95% of the patients received ACE and/or ATRA. Baseline systolic BP was 140.3 ± 18.9 mmHg (no CCB) and 144.8 ± 16.4 mmHg (CCB); diastolic BP was 81.7 ± 6.6 mmHg (no CCB) and 75.8 ± 6.9 mmHg (CCB); proteinuria was 2.7 ± 3.1 g/day (no CCB) and 2.6 ± 2.9 g/day (CCB). The two groups did not differ significantly in their usage of ACEi and/or ATRA, baseline BP, and proteinuria ($p = 0.841$). At 12 months, there was significant reduction in systolic ($p < 0.0001$) and diastolic BP ($p < 0.001$) in the overall cohort with no between group differences. Proteinuria was reduced to 2.3 ± 3.5 g/day (no CCB) and 2.2 ± 2.9 g/day (CCB) respectively. There was no difference in the degree of proteinuria reduction between the two groups ($p = 0.943$). Repeat analysis with the diabetic sub-group revealed similar findings.
Conclusion: Use of DHCCB did not affect the degree of proteinuria reduction given similar degree of BP reduction.

THEME 3: INFLAMMATION AND IMMUNOLOGY

Adhesion Molecules

W-PO30001

ROLES OF AND CORRELATION BETWEEN ÉØ-SMOOTH MUSCLE ACTIN, CD44, HYALURONIC ACID AND OSTEOPONTIN IN CRESCENT FORMATION IN HUMAN GLOMERULONEPHRITIS

HIRONORI NAKAMURA[1], KOZO KITAZAWA[1], HIROKAZU HONDA[1], TETSUZO SUGISAKI[1]
Department of Nephrology, Showa University School of Medicine[1]

Background: In a rat model, myofibroblasts, which express Éø-smooth muscle actin (SMA), CD44, hyaluronic acid (HA) and osteopontin (OPN), are involved in crescent formation. However, the roles of the molecules and interactions involved in crescent formation and evolution in human glomerulonephritis are unknown.
Methods: This study was performed to examine the expressions of Éø-SMA, CD44, HA, OPN and CD68 by immunohistochemistry in 14 patients with crescentic glomerulonephritis. Crescents were classified into cellular, fibrocellular and fibrous. The extent of staining in each crescent was scored semiquantitatively. The change and correlation of expression of each molecule during crescent formation and evolution were determined statistically.
Results: There was strong CD44, OPN and CD68 staining in the cellular crescent, whose expressions decreased in the fibrocellular crescent and were almost negative in the fibrous crescent. There were significant differences between the expressions of CD44, HA, OPN and CD68 in the cellular crescent compared with those in the fibrous crescent. CD44 expression from the cellular crescent to the fibrous crescent correlated with the expressions of OPN, CD68 and HA. A large extent of HA deposition was detected in the cellular crescent, which decreased in the fibrocellular crescent. A large number of Éø-SMA-positive cells were observed in the fibrocellular crescent, and some of Éø-SMA-positive cells showed an elongated morphology. There was a significant difference in the expression of Éø-SMA in the fibrocellular crescent compared with that in the cellular crescent.
Conclusion: The present study has demonstrated the marked up-regulation of CD44, HA, OPN and CD68 at the early stage of the development of human crescentic glomerulonephritis. Moreover, we conclude that myofibroblasts and cell-matrix interactions mediated by the CD44-OPN and CD44-HA receptor-ligand pairs may play important roles in the progression of crescent formation.

W-PO30002

INVOLVEMENT OF JUNCTIONAL ADHESION MOLECULE (JAM) 2 AND 3 IN ANTI-GLOMERULAR BASEMENT MEMBRANE (GBM) GLOMERULONEPHRITIS (GN) MICE

FANG LIU[1], SONIA CUNNINGHAM[2], WANSHENG WANG[2], XIAO R. HUANG[2], LUAN TRUONG[2], HUI Y. LAN[2]
Division of Nephrology, Department of Medicine, West China Hospital of Sichuan University, Chengdu, Sichuan, China 610041[1], Department of Medicine, Section of Nephrology and Leukocyte Biology and Pathology, Baylor College of Medicine, Houston, TX 77030. USA[2]

JAMs are differentially expressed by the endothelium and various leukocyte subsets. Current evidences implicate their active role during leukocyte transendothelial migration, with JAM3 serving as an adhesive counter-receptor for JAM2. The aim of this study was to examine whether JAM2 and JAM3 were involved in anti-GBM GN mice. Immunohistochemistry showed that both JAM2 and JAM3 were weakly expressed in glomerular endothelial cells of normal mouse kidney. However, JAM3 protein was rapidly upregulated in the glomeruli 3 hours after disease induction (a 2-fold increase over normal) and peaked at day 1 (a 3.8-fold increase over normal), then decreased at day 7, while increased by 13.7 folds in tubulointerstitial endothelial cells at day 7. JAM2 protein expression increased at day 7. The upregulation of JAM2 and JAM3 were associated with glomerular crescentic formation at day 7. Though JAM2 and JAM3 proteins were markedly increased in the diseased kidney, there was no change in JAM2 and JAM3 mRNA levels throughout the experimental period by real-time PCR. *In Situ* hybridization analysis further confirmed that JAM2 and JAM3 mRNA expression in glomeruli were consistent with their protein expression levels. Correlation analysis showed that expression of JAM2 and JAM3 were correlated with glomerular and interstitial macrophages infiltration and interstitial T cells accumulation. In conclusion, JAM2 and JAM3 are upregulated in anti-GBM GN, which may be associated with the recruitment of T cells and macrophages to the sites of renal inflammation.

W-PO30003

SYNERGISTIC INDUCTION OF MCP-1 BY INTEGRINS AND PDGF

MASAHITO TAMURA[1], NARUTOSHI KABASHIMA[2], MASAKI TOKUNAGA[2], HIROFUMI ANAI[2], KAORI KANEGAE[2], RYOTA SERINO[2], HIROSHI MATSUO[2], TATSUYA SHIBATA[2], MIKA MATSUMOTO[2], YASUHIDE NAKASHIMA[2]
Kidney Center, University of Occupational and Environmental University Hospital, Japanl[1], Second Department of Internal Medicine[2]

Growth factors and ECM as well as its receptor integrins are upregulated in many types of human and experimental glomerular diseases, however, little is known regarding interaction between integrin- and growth factor-mediated intracellular signalings in the regulation of gene expression involved in the progression of chronic kidney diseases. We examined for interaction between integrin- and PDGF-mediated signalings involving focal adhesion kinase (FAK) and Erk 1/2 pathways that lead to MCP-1 expression in cultured rat mesangial cells (MC). Cell adhesion to fibronectin increased phosphorylation of not only FAK but also MEK 1/2 and Erk 1/2 and induced MCP-1 mRNA and protein expression in the absence of PDGF. PDGF increased phosphorylation of FAK as well as MEK 1/2 and Erk 1/2 even without cell adhesion to ECM and induced MCP-1 mRNA and protein expression. Simultaneous stimulation of cells by both fibronectin and PDGF enhanced phosphorylation of FAK, MEK 1/2, and Erk1/2. Synergism for MCP-1 mRNA and protein expression was also observed between PDGF and fibronectin. Transfection of dominant-negative FAK attenuated fibronectin enhancement of PDGF-induced Erk1/2 phosphorylation and MCP-1 expression, indicating involvement of FAK in this signaling. These results demonstrate the cross talk between integrin and PDGF signalings at the level of FAK in MC, and that adhesion to FAK is required for maximal induction of PDGF-induced MAP kinase activation. The collaboration between integrin and PDGF signalings may contribute to progression of glomerular diseases through the induction of MCP-1.

W-PO30004

INTEGRIN-MEDIATED INDUCTION OF ICAM-1 IN HUMAN MESANGIAL CELLS

MASAHITO TAMURA[1], MASAKI TOKUNAGA[1], NARUTOSHI KABASHIMA[1], SHINGO NAKAYAMADA[2], YOSHIYA TANAKA[2], HIROFUMI ANAI[2], RYOTA SERINO[2], HIROSHI MATSUO[2], MIKA MATSUMOTO[2], TATSUYA SHIBATA[2], YASUHIDE NAKASHIMA[2]
Kidney Center University of Occupational and Environmental Health University Hospital[1], Second Department of Internal Medicine[2]

Integrins are major adhesion receptors that regulate cytoskeletal organization and motility. They also trigger a variety of signal transduction pathways and gene expression. We examined the modulatory effects of ECM protein accumulation observed in various types of glomerulopathies on the expression of genes involved in the progression of renal diseases. Quiescent human mesangial cells abundantly expressed cell surface molecules such as alpha 2, 3, 4, 5 and beta 1 integrins and CD44, weakly alpha 6 and v and beta 3 integrins, ICAM-1, and Fas, and no alpha 1 and beta 2 integrins and VCAM-1 examined by FACS analysis. Stimulation of beta 1 integrin induced time-dependent expression of ICAM-1, but not of Fas or VCAM-1. Stimulation of MHC class I, ICAM-1, and VCAM-1 did not alter expression levels of ICAM-1, Fas, and VCAM-1. Cell adhesion to type I or III collagens, which have affinity for alpha 2 beta 1 integrin, enhanced ICAM-1 expression, however, adhesion to fibronectin, laminin, type IV collagens, and non-specific adhesion substrates such as concanavalin A, polylysine, and albumin had no effect on ICAM-1 expression. Furthermore, pretreatment of cells with anti-alpha 2 integrin inhibitory antibody completely inhibited beta 1 integrin-induced expression of ICAM-1, although antibodies against alpha 1, 5, and v integrins failed to block ICAM-1 expression. Similarly, type I and III collagens-induced expression of ICAM-1 was suppressed by pretreatment of cells with anti-alpha 2 antibody. Treatment of cells with MEK inhibitor, tyrosin kinase inhibitor, or protein kinase C (PKC) inhibitor suppressed levels of beta 1 integrin-induced MCP-1 mRNA expression. Our results indicate that stimulation of alpha 2 beta 1 integrin induces ICAM-1 expression through tyrosin kinases and PKC, and suggest that accumulation of pathological ECM components such as type I and III collagens might play a role in the progression of glomerular diseases by enhancing ICAM-1 expression.

W-PO30005

L1 CELL ADHESION MOLECULE: A TISSUE AND URINARY BIOMARKER FOR ACUTE TUBULAR NECROSIS (ATN) IN DISTAL NEPHRON AND COLLECTING DUCT

HANNA DEBIEC[1], YVES ALLORY[2], FREDERIC COMMO[3], PHILIPPE FONTANGES[2], PIERRE RONCO[2]
INSERM U702, Tenon hospital, France[1], Department of Pathology, Tenon hospital, France[2]

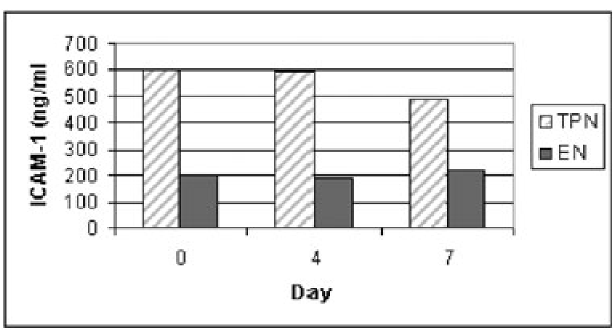
L1 cell adhesion molecule is a transmembrane glycoprotein of the immunoglobulin superfamily. Here, we evaluated whether L1 expression is changed in human ATN and may serve as a tissue and urinary marker of acute renal tubular injury. Using immunohistochemistry method with two antibodies to the extracellular part and the cytoplasmic domain, respectively, we first compared L1 expression in normal kidney, and in series of 20 human biopsies from naive and transplant kidneys during ATN. Then we studied L1 expression in those biopsies together with markers of differentiation, cell polarity, and proliferation. Urine samples were collected from additional 30 patients with ATN for detection of extra- and intracytoplasmic domains of the L1 protein by Western blot analysis. In normal kidney, L1 immunoreactive with both antibodies was expressed at the basolateral or lateral side in all epithelial cells in collecting duct except for intercalated cells. In ATN, L1 was detected in the basolateral and apical domains in collecting duct and in addition, was induced in thick ascending loop and distal tubules. L1 apical expression during ATN was not related to a loss of polarity in collecting duct epithelium as evidenced by normal localization of cell polarity markers. In most severe ATN, apically expressed L1 reacted only with the antibody to intracytoplasmic domain, suggesting that the protein was cleaved. Quantitative Western blot analysis of urines revealed that the extracytoplasmic part of L1 appeared in the urine of patients with ATN. In conclusion, a soluble form of human L1 can be detected in the urine of patients with ATN and may serve as a biomarker for renal distal nephron and collecting duct injury.

W-PO30006

THE RELATIONSHIP OF THE ROUTE OF NUTRITION AND ADHESION MOLECULES AND THEIR PROGNOSTIC IMPLICATIONS IN ARF PATIENTS

MEHMET SEZER[1], GOKHAN GUNGOR[2], MURAT DEMIR[3], FATIH GULTEKIN[1], OZLEM OZTURK[1]
Suleyman Demirel University School Of Medicine[1], Internal Medicine, Suleyman Demirel University School Of Medicine, Turkey[2], Nephrology, Suleyman Demirel University School Of Medicine, Turkey[3]

Increased intestinal intercellular adhesion molecule-1 (ICAM-1) is shown with parenteral nutrition. In a clinical study, it is detected that plasma ICAM-1 concentrations independently predict number of organs failed, development of more than three organ failure and mortality. Therefore we wanted to compare serum ICAM-1 levels, infection and mortality rates, specifically in patients with acute renal failure (ARF), who receiving enteral (EN) or parenteral nutrition (TPN). Sixty-four patients with ARF who needed nutritional support were recruited for the study (mean age, 64.1 ± 12.3 years; 34 M, 30 F). Blood samples for ICAM-1 were drawn at the first nephrology consult day, days 4 and 7 of follow-up. ICAM-1 level was measured by ELISA. If both types of nutrition routes were used in a patient during hospitalization, these patients were excluded from the study. Forty-five EN and 19 TPN patients were compared for serum ICAM-1, albumin levels, infection and in hospital mortality rates.
Demographic characteristics were similar in both groups. The rates of oliguric and/or dialyzed patients were not statistically different. APACHE III score was a little bit higher in TPN group ($p = 0.053$). Nevertheless, serum ICAM-1 levels, infection and mortality rates were higher and baseline serum albumin levels were lower in TPN group than in those of EN group ($p < 0.001$, $p = 0.001$, $p = 0.049$ and $p = 0.01$, respectively). There was also a negative correlation between baseline serum albumin and baseline serum ICAM-1 levels ($r = -0.36$, $p = 0.003$).
In conclusion, this study shows that serum ICAM-1 levels in total parenteral nutrished ARF patients were higher those in EN group and also increased serum ICAM-1 level was associated with increased infection and mortality rates. Therefore, we suggest that EN should be the main nutrition modality as possible as in patients with ARF. Also, further studies are required to clarify causal relationship between increased ICAM-1 levels and higher mortality rates in patients receiving TPN.



W-PO30007

RAPAMYCIN AFFECTS B1 INTEGRINS EXPRESSION AND ILK FUNCTION IN RENAL CELL CARCINOMA (RCC)

MARILISA SALDARELLI[1], LUIGI BALDUCCI[2], VITO MANCINI[3], PASQUALE DITONNO[3], MICHELE BATTAGLIA[3], PAOLA PONTRELLI[4], GIUSEPPE GRANDALIANO[4], FRANCESCO PAOLO SCHENA[4], LORETO GESUALDO[5], ELENA RANIERI[6]

Department Of Emergency And Organ Transplantation, Section Of Nephrology, University Of Bari, Italy[1], Dept. Of Surgery, University Of Foggia, Italy[2], DETO, Section Of Urology, University Of Bari, Italy[3], DETO, Section Of Nephrology, University Of Bari, Italy[4], Dept. Of Biomedical Sciences, Section Of Nephrology, University Of Foggia, Italy[5], Clinican Pathology, University Of Foggia, Italy[6]

RCC is an important cause of morbidity and mortality, and its incidence is increasing. Rapamycin is an immunosoppressor that inactivates mammalian target of rapamycin (mTOR). Rapamycin has shown to inhibit the growth a variety of tumor cell lines. The integrin cytoplasmic domain modulates several cellular processes. Five different splicing variants have been identified for b1 integrin; in particular, b1C and b1A differentially affect cell proliferation: b1C inhibits proliferation, whereas b1A promotes it. Integrin-linked kinase (ILK) is an intracellular protein, which interacts with the cytoplasmic domain of integrin b1.

The aim of the study was to investigate rapamycin effects on b1 integrins expression and ILK/Akt/mTOR signaling pathway in normal and RCC cell lines. These studies evidenced a modulation of p-mTOR but not Akt in a time-dependent manner. Rapamycin (20 ng/ml) induced a significant decrease in cellular p-mTOR, peaking at 20 min and returning to basal levels after 60 min of incubation. Real Time PCR demonstrated that b1C and b1A mRNA levels were reduced in RCC cell lines (-5 and -4 fold decrease over normal cells). Stimulation of cancer cells with 20 ng/ml rapamycin has been shown to further decrease the b1 splice variants mRNA levels (b1C 2 fold decrease over basal and b1A 1,5 fold decrease over basal).

We evaluated b1 protein levels by western blotting. Rapamycin treatment was able to notable increase b1 integrin in cancer cell lines versus normal cell lines. Immunoblotting demonstrated that could exist a transcriptional and/or translational regulation.

We next evaluated ILK protein levels. Immunoblotting demonstrated that in cancer cells ILK protein levels were increased versus normal renal cells, and rapamycin treatment highlighted a modulation of gene expression in a time-dependent manner (decrease between 10–20 min). In conclusion, this is the first report of rapamycin effects on b1 integrin expression in normal and cancer renal cells.

Growth Factors

W-PO30008

EVALUATION OF INSULIN LIKE GROWTH FACTOR 1 IN CHILDREN WITH DIFFERENT STAGES OF CHRONIC RENAL FAILURE

ALI DERAKHSHAN[1], HAMDOLLAH KARAMIFAR[1], SEYYED MOSTAJAB RAZAVI[2], MOHAMMAD HOSSEIN FALLAHZADEH[1], MITRA BASIRATNIA[1], GHAMAR HOSSEINI AL-HASHEMI[1]

Shiraz University of Medical Sciences[1], Shiraz Health Center[2]

Children with chronic renal failure (CRF) fail to attain adult height consistant with their genetic potential. Growth failure in these children is multifactorial: some of the major contributing factors are as follows: low caloric intake, metabolic acidosis, renal osteodystrophy, anemia, drug toxicity, uremic toxins and inhibitors of hormone action. Also abnormalities of the growth hormone/insulin-like growth factor (IGF) axis in CRF may contribute to this poor growth. Aim of this study was evaluation of serum IGF1 in dialysis and predialysis children with CRF compared with normal children.

We select four groups of children up to 18 y/o, 22 cases of CRF that was on regular hemodialysis, 26 cases of CRF with different glumerular filtration rate (GFR) in predialysis state, 23 children with normal height and weight for age and 23 children with constitional short stature. Serum level of Insulin like growth factor I (IGF-1) was determined in all groups. SPSS software used for statistical analysis. Mean serum level of IGF in dialysis children (Group one) was: 209.26 ± 141.65 ng/ml and in predialysis children with chronic renal failure was 159.29 ± 163.9 ng/ml. In normal children mean serum level of IGF1 was 420.5 ± 182 ng/ml and in children with constitutional short stature was 360.9 ± 183.3 ng/ml. The results showed that serum level of IGF-1 in CRF was significantly lower compared with normal children, both in dialysis and non-dialysis CRF ($P < 0.0001$). There was no statistical difference in dialysis and nondialysis group ($P = 0.449$). No correlation was found between IGF-I and glomerular filteration rate in group one ($P = 0.179$) and group two ($P = 0.305$).

In conclusion, serum level of IGF1 in dialysis and predialysis children were significantly lower than normal children.

W-PO30009

P42/44 MAPK MEDIATES PRODUCTIONS OF CHEMOKINES BY MESANGIAL CELLS STIMULATED BY CONNECTIVE TISSUE GROWTH FACTOR

SHENG-HUA WU[1], CHAO LU[1], LING DONG[1], GUO-PING ZHOU[1]

Department of Pediatrics, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China[1]

To examine whether connective tissue growth factor (CTGF) induced the productions of chemokines by glomerular mesangial cells of rats, the mRNA expression of fractalkine, monocyte chemoattractant protein-1 (MCP-1) and regulated on activation, normal T cell expressed and secreted (RANTES) were analyzed by reverse transcription (RT)-PCR in cultured mesangial cells stimulated by CTGF. The proteins of fractalkine, MCP-1 and RANTES in the supernatants of cells were determined by enzyme-linked immunosorbent assay (ELISA). The chemotactic effect of the supernatants on monocytes was assessed by the in vitro chemotaxis assay. The phosphorylation of p42/44 MAPK was assessed by Western blotting analysis. Treatment of the cells with CTGF enhanced the mRNA expressions of fractalkine, MCP-1 and RANTES and concentrations of fractalkine, MCP-1 and RANTES in the supernatants. Pretreatment of the supernatants of CTGF-treated cells with anti-fractalkine, anti-MCP-1 and anti-RANTES antibodies partially inhibited the chemotactic effects of the supernatants on monocytes respectively. CTGF increased the expression of phospho (P)-p42/44MAPK in mesangial cells. Pretreatment of the cells with PD98059, an inhibitor of P-p42/44 MAKP, blocked the CTGF-induced expression of p42/44 MAPK, partially inhibited the concentrations of fractalkine, MCP-1 and RANTES in supernatants of mesangial cells stimulated by CTGF. In conclusion, CTGF induced the secretion of fractalkine, MCP-1 and RANTES by mesangial cells via the mechanisms of p24/p44 MAPK phosphorylation dependent pathway.

W-PO30010

ERK-DEPENDENT CELL PROLIFERATION IN THE OBSTRUCTED MOUSE KIDNEY

YINGJIE HAN[1], TAKAO MASAKI[1], LYNETTE A. HURST[1], YOHEI IKEZUMI[1], JAMES M. TRZASKOS[2], ROBERT C. ATKINS[3], DAVID J. NIKOLIC-PATERSON[3]

Department of Nephrology, Monash Medical Centre, Clayton, Victoria 3168, Australia[1], Immunology Biology, Bristol-Myers Squibb Co., Pharmaceutical Research Institute, Princeton, NJ, USA[2], Department of Nephrology and Monash University Department of Medicine, Monash Medical Centre[3]

A number of growth factors have been shown to induce proliferation of renal cell types in animal models of kidney disease. In vitro studies suggest that these growth factors induce renal cell proliferation through the MEK1-ERK signalling pathway. The aim of this study was to determine the functional role of ERK signalling in cell proliferation in the obstructed kidney. Unilateral ureteric obstruction (UUO) was induced in C57BL/6J mice which then received the MEK1 inhibitor (U0126 at 100 mg/kg U0126, tid), vehicle (DMSO), or no treatment (no Tx) starting at day 2 after UUO surgery and continuing until animals were killed on day 5. Cell proliferation was assessed by injection of bromodeoxyuridine (BrdU) 3 hours before animals were killed. In normal mice, activation (phosphorylation) of ERK was restricted to collecting ducts. Western blotting identified a marked increase in p-ERK in the obstructed kidney in the no Tx and vehicle treated groups. Immunostaining showed strong p-ERK staining in many tubules and interstitial cells. U0126 treatment inhibited MEK1 activity as shown by an almost complete loss of ERK phosphorylation as assessed by Western blot and immunostaining. The number of BrdU+ cortical tubular cells was reduced by vehicle treatment but was not further changed by U0126 treatment (15.2 ± 4.4, 9.2 ± 4.8 and 8.6 ± 3.4 BrdU+ tubular cells/hpf for no Tx, Veh and U0126, respectively). In contrast, interstitial cell proliferation in the obstructed kidney was unaltered by vehicle treatment (12.2 ± 4.9 vs 11.0 ± 6.8 BrdU+ interst cells/hpf), but was this was significantly inhibited by U0126 (3.9 ± 3.6 BrdU+

interst cells/hpf; $P < 0.05$ vs both control groups). This was associated with a reduction in interstitial macrophage accumulation, but no effect was seen upon interstitial accumulation of SMA+ myofibroblasts. In summary, these studies have shown that proliferation of cortical interstitial cells in the obstructed kidney operates through the ERK signalling pathway.

W-PO30011

TGF-BETA1 UP-REGULATES SHARP-2 GENE EXPRESSION VIA PI-3 KINASE PATHWAY IN LLC-PK1

ZHANGFEI SHOU[1], JIANGHUA CHEN[1], QIANG HE[1], YAMADA KAZUYA[2], MIYAMOTO KAORU[2]
The Kidney Disease Center, The First Affiliated Hospital, Medical College, Zhejiang University, Hangzhou, China[1], Department Of Biochemistry, Fukui Medical University[2]

Background: The rat enhancer of split- and hairy-related protein-2 (SHARP-2) is a basic helix-loop-helix transcription factor, it plays several critical roles of cell function. We have determined that TGF-beta1 can induce a 3-fold expression of SHARP-2 gene in renal epithelial tubular cell lines (LLC-PK1) cells. TGF-beta1 plays key role in the fibrosis leading to ESRD. SHARP-2 is a novel down stream target of TGF-beta1, therefore the determination of the pathway of TGF-beta1 inducing the expression of SHARP-2 will shed lights to the understanding of some kidney disease. Purpose: To determine the pathway that contributed to the induction of SHARP-2 gene expression with TGF-beta1 stimulation.
Methods: Northern blot analysis were used to investigate the expression of SHARP-2 gene. Several chemical inhibitors, including PD98059, Wortmannin, LY294002, AG-490, Okadaic acid, Rapamycin etc, were employed. We also used an adenovirals that stably express PI-3K to verify the pathway determined by the chemical inhibitor method.
Results: Wortmannin, and LY294002 blocked the induction with a dose-dependent manner. Resulds from the PI-3K-expressing adenovirus experiment confirmed the pathway determined.
Conclusion: PI-3 kinase pathway contributed to the up-regulation of SHARP-2 gene expression with TGF-beta1 stimulation. Generally, TGF-beta1 signaling via Smad pathway. Our experiment founded a novel signaling pathway of TGF-beta1.

W-PO30012

SELECTIVE INHIBITION OF TGF-BETA TYPE I RECEPTOR (ALK5) AMELIORATES RENAL TUBULOINTERSTITIAL FIBROSIS

HIDEKI YOKOI[1], MASASHI MUKOYAMA[2], MANABU NAGATA[2], KAZUTOMO SAWAI[2], TETSUNO YOSHIOKA[2], YOKO SAITO[2], TETSUYA NAGAE[2], AKIRA SUGAWARA[2], KAZUWA NAKAO[2]
Kyoto University Graduate School of Medicine[1], National Hospital Organization Kyoto Medical Center[2]

Tubulointerstitial fibrosis is a common feature of progressive renal injury in almost all forms of renal diseases. Among proposed mechanisms by which the renal injury progresses, TGF-beta is postulated to play a central role in the development of tubulointerstitial fibrosis. TGF-beta acts through the TGF-beta type I (ALK5) and type II receptors to activate intracellular mediators, such as Smad proteins. Selective inhibitor of ALK5 has been reported to reduce TGF-beta-induced fibronectin and type I collagen expression in cultured cells. However, the effects of ALK5 inhibitor in renal tubulointerstitial fibrosis in vivo remains unclear. In the present study, we investigated the effects of ALK5 inhibitor on extracellular matrix production and interstitial fibrosis in mice kidneys with unilateral ureteral obstruction (UUO). Male C57BL/6J mice were treated with ALK5 inhibitor (100 mg/kg/day) or vehicle after UUO for 14 days. Immunohistochemical study for phosphorylated Smad2/3 (p-Smad2/3) showed that the number of positive staining nuclei in the interstitial and the renal tubular cells in ALK5-treated mice kidneys decreased compared with vehicle-treated mice kidneys. ALK5 treatment also reduced the gene expression of CTGF and fibronectin in the obstructed kidneys compared with vehicle-treated mice. The deposition of fibronectin and type I collagen and the interstitial fibrotic areas in ALK5-treated mice were markedly attenuated. These findings indicate that ALK5 inhibitor reduces extracellular matrix production and ameliorates interstitial fibrosis during UUO. ALK5 inhibitor could be a new therapeutic drug against renal fibrosis.

W-PO30013

NEW PLASMATIC GROWTH FACTOR SYSTEM IN CHRONIC KIDNEY DISEASE PATIENTS

JACEK BORAWSKI[1], BEATA NAUMNIK[1], ALICJA RYDZEWSKA-ROSOŁOWSKA[1], KRYSTYNA PAWLAK[1], SZYMON BRZOSKO[1], LECH CHYCZEWSKI[2], MICHAŁ MYŚLIWIEC[1]
Dept. of Nephrology and Transplantology with Dialysis Unit, Medical University, Bialystok, Poland[1], Dept. of Clinical, Molecular Biology, Medical University, Bialystok, Poland[2]

Hepatocyte growth factor (HGF), activin A (Act A), its main inhibitor follistatin (FS), and transforming growth factor-β1 (TGF-β1) are potent, pleiotropic and of growing significance in the development, regeneration and repair of almost all tissues and organs. Their plasma levels were measured with commercial ELISA kits in 4 groups of clinically-stable CKD patients (n = 182; in maintenance HD patients – before heparin administration) and compared with those in 30 healthy subjects.

	HD n = 35	CAPD n = 38	TX n = 55	CKD 1-4 n = 54	Controls n = 30
HGF, *ng/ml*	1.89[a] (0.93–11.6)	1.18[b] (0.60–4.09)	0.78 (0.53–2.70)	0.68 (0.23–11.2)	0.58 ±0.15
Act A, *ng/ml*	0.98[c] (0.56–2.56)	1.29[c] (0.42–3.30)	0.44[d] (0.19–5.13)	0.45[d] (0.22–1.70)	0.29 ±0.13
FS, *ng/ml*	2.49[e] (0.54–7.17)	1.46 (0.64–4.97)	1.53 (0.74–3.90)	1.60 (0.31–7.61)	1.20 (0.57–2.82)
Act A/FS ratio	0.40 (0.11–1.71)	0.70[f] (0.09–4.31)	0.32 (0.10–2.81)	0.31 (0.04–1.48)	0.22 (0.04–0.77)
Free TGF-β1, *ng/ml*	6.64 ±2.47	7.59[g] (4.21–31.0)	4.73 (2.01–28.4)	10.6[h] (1.58–72.4)	3.28 (1.26–8.95)

[a] $P < 0.0002$ *vs* others; [b] $P < 0.05$ *vs* TX, CKD 1-4 and Controls; [c] $P < 0.0001$ *vs* TX, CKD 1-4 and Controls; [d] $P < 0.02$ *vs* Controls; [e] $P < 0.002$ *vs* others; [f] $P < 0.0001$ *vs* others; [g] $P = 0.0007$ *vs* Controls; [h] $P < 0.003$ *vs* others.

In addition to the different plasma levels, the growth factors also demonstrated diverse but biologically-consistent and clinically-plausible patterns of reciprocal relations (data not shown). These suggest, for the first time, existence of plasmatic cytokine networks that are specific for each of the renal patient population. In conclusion, complex systemic activation of pleiotropic growth factors/cytokines such as HGF, Act A, FS and TGF-β1 is remarkable in CKD patients and depends on stage of the disease and modality of renal replacement therapy.

W-PO30014

THE ROLE OF CONNECTIVE TISSUE GROWTH FACTOR IN EPITHELIAL-MYOFIBROBLAST TRANSDIFFERENTIATION OF RENAL TUBULAR CELL

JINGPING XING[1], NAN CHEN[1], WEIMING WANG[1], JIAN LU[2]
Department of Nephrology, Ruijin Hospital, Shanghai Second Medical University, Shanghai 200025 P.R. China[1], Department of Biochemistry and Molecular Biology, Shanghai Second Medical University, Shanghai 200025, China[2]

Background: Connective tissue growth factor (CTGF) is one of the candidate factors mediating downstream events of transforming growth factor-beta1 (TGF-β1), but whether itself alone can induce tubulointerstitial fibrosis, especially epithelial-myofibroblast transdifferentiation, has not yet been clearly identified.
Methods: PcDNA3.1/Zeocin-CTGF plasmid was transfected into cultured human proximal renal tubular epithelial cells (HK-2 cells) and Zeocin (400 ug/ml) was added to the culture medium to gain the HK-2 cell lines stably expressing CTGF. RT-PCR and Western Blot analysis were used to confirm the expression of CTGF in HK-2 cells. In addition, we stimulated HK-2 cell stably expressing CTGF with 5 ng/ml TGF-β1 to identify if TGF-β1 and CTGF have a synergistic effect on epithelial-myofibroblast transdifferentiation.
Results: Normal HK-2 cells express detectable CTGF mRNA and protein. HK-2 cells stably transfected with CTGF express high levels of CTGF. Although HK-2 cells stably transfected with CTGF have no significant change in cell shape, their expression of alpha-smooth muscle actin was augmented. Moreover, the tubular epithelial cells transfected with CTGF did not increase the expression of type I collagen, when compared with control plasmid (pcDNA3.1/Zeocin) transfection group. Twenty-four hours after TGF-β1 stimulation, the HK-2 cells stably expressing CTGF showed obvious change in cell shape (shuttle shape), which was early than that in control group (48 hours). While forty-eight hours after

TGF-β1 stimulation, the expression of alpha-smooth muscle actin detected by Western Blot analysis was much more in HK-2 cells stably expressing CTGF than in control plasmid transfection cells.
Conclusions: CTGF may have a major role in inducing epithelial-myofibroblast transdifferentiation while having little contribution to extracellular matrix deposition. CTGF and TGF-β1 can work synergistically to induce epithelial-myofibroblast transdifferentiation.

W-PO30015

THROMBOSPONDIN-1INDUCED BY ANGIOTENSIN II IS A MAJOR ACTIVATOR OF LATENT TGF-β1 IN HUMAN RENAL TUBULAR EPITHELIAL CELL

KAIYIN WU[1], WANG WEIMING[1], ZHANG BIN[1], CHEN NAN[1]
Department of Nephrology, Ruijin Hospital, Shanghai Second Medical University, Shanghai 200025, PR China[1]

Angiotensin II (ANG II) induces secretion and activation of transforming growth factor-β1 (TGF-β1) by renal tubular epithelial cells (TECs). However, the mechanisms that operate this are unclear. Thrombospondin-1 (TSP-1), which is produced by TECs in damaged tubularinterstitium, is one of several molecules known to activate the latent TGF-β1 complex. Therefore, we examined whether the ANG II-induced activation of latent TGF-β1 in TECs operates via TSP-1.
Methods: ANG II (100 nM) stimulates human renal tubular epithelial cell line(HK-2) for 24 hours with the pre-treatment of ANG II type 1 (AT1)-receptor antagonist(Losartan 10 uM) or TSP-1-specific blocking peptide (GGWSHW 10 uM), which was known to block the interaction between TSP1 and latent TGF-β1 complex. The TSP1/TGF-β1 protein production was detected by flow cytometry and Western blot analysis. The expression of fibronectin and p-smad2 were also measured by Western blot. Con-focal microscopy was used to show the presentation of TSP1, TGF-β1, fibronectin and α-SMA, as the mRNA encoding level of above proteins were followed by RT-PCR.
Results: ANG II significantly increased TSP-1, TGF-β1, fibronectin, α-SMA and p-smad2 mRNA and protein production in HK-2 cells. Production of ANG II-induced TSP-1 mRNA and protein was partly inhibited by Losartan but was unaffected by blocking peptide (GGWSHW). Use of losartan and hexo-peptides can decrease the ANG II induced activation of latent TGF-β1, which was demonstrated by the down-regulation of fibronectin and p-smad2.
Conclusion: Our study has demonstrated that ANG II-induced activation of latent TGF-β1 in HK-2 operates via TSP-1. Furthermore, ANG II-induced TSP-1 production is dependent on angiotensin II type 1 receptor.

Signaling Factors

W-PO30016

OREXIN-A, A NOVEL LOCAL REGULATOR IN THE KIDNEY

KAZUHIRO TAKAHASHI[1], ZENEI ARIHARA[2], KAZUHITO TOTSUNE[3], MASAHIKO SONE[4], KUMI KIKUCHI[2], OSAMU MURAKAMI[5]
Department of Molecular Biology & Applied Physiology, Tohoku University School of Medicine, Sendai, Japan[1], Department of Medicine[2], Department of Clinical Pharmacology &Therapeutics[3], Department of Applied Physiology & Molecular Biology[4], Department of Medicine Tohoku University Schools of Medicine and Pharmaceutical Science[5]

Orexin-A, a neuropeptide with stimulatory actions on arousal and appetite, was originally shown to be specifically expressed in the hypothalamus. We studied expression of orexin-A and orexin receptors in the kidney and the presence of orexin-A-like immunoreactivity in human urine. Immunocytochemistry showed that orexin-A-like immunoreactivity was localized in the tubules of the human kidney obtained at autopsy. Orexin-A-like immunoreactivity was detected in human kidneys (15.1 & 15.7 fmol/g wet weight, n = 2) and rat kidneys (16.2 +/– 1.6 fmol/g wet weight, mean +/– SEM, n = 5) by radioimmunoassay, although the levels were much lower than the levels in the brain. Orexin-A-like immunoreactivity was present in the urine obtained from male healthy volunteers (67.8 +/– 4.5 pmol/L, n = 5). Reverse phase high performance liquid chromatography showed that most of orexin-A-like immunoreactivity of the urine extract was eluted earlier than authentic orexin-A, suggesting that orexin-A-like immunoreactivity in urine was modified to hydrophilic forms. Reverse transcriptase polymerase chain reaction showed expression of orexin receptor 1 and 2 mRNAs in the human kidney. These findings suggest that orexin-A is produced by the renal tubular cells and secreted into urine. Orexin-A may act on the kidney in the autocrine or paracrine fashion, or via the urine (urocrine fashion).

W-PO30017

ENDOGENOUS PROSTAGLANDIN D2 SYNTHESIS INHIBITS PLATELET-DERIVED GROWTH FACTOR GENERATION IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

HIDEYUKI NEGORO[1], YOSHIO UEHARA[1], RIE TAGUCHI[1], ATSUO GOTO[1], KEISUKE NISHIYAMA[1], TERUHIKO TOYO-OKA[1], MASAO OMATA[1], TOSHIRO FUJITA[1]
University of Tokyo[1]

Objective: We examined the role of prostaglandin D2 (PGD2) in the formation of platelet-derived growth factor (PDGF) following interleukin-1 (IL-1) stimulation in human umbilical vein endothelial cells (HUVEC) transfected with lipocaline-type PGD2 synthase (L-PGDS) genes.
Design and Methods: HUVEC were isolated from human umbilical vein and incubated with 20 U/ml IL-1 and various concentrations of authentic PGD2. The isolated HUVEC were also transfected with L-PGDS genes by electroporation. The L-PGDS-transfected HUVEC were used to investigate the role of endogenous PGD2 in IL-1-stimulated PDGF biosynthesis. We also used an anti-PGD2 antibody to examine whether an intracrine mechanism was involved in PDGF production. PGD2 and PDGF-AA/AB levels were determined by radio- and enzyme-immunoassay, respectively. PDGF-A/B mRNA was assessed by RT-PCR.
Results: IL-1-stimulated PDGF production by HUVEC was dose-dependently inhibited by authentic PGD2 at concentrations greater than 10–6 mol/l. L-PGDS gene-transfected HUVEC produced more PGD2 than HUVEC transfected with the reporter gene alone. IL-1 induced increases in PDGF-AA/AB production in HUVEC transfected with reporter genes alone. However this effect was significantly attenuated in the case of IL-1 stimulation of HUVEC transfected with L-PGDS genes, and accompanied by an apparent suppression of PDGF-A/B mRNA expression. Neutralization of extracellular PGD2 by anti-PGD2-specific antibody influenced neither PDGF-A/B mRNA expression nor PDGF-AA/AB biosynthesis.
Conclusions: HUVEC transfected with L-PGDS genes showed increased PGD2 synthesis. This increase was associated with attenuation of both PDGF generation and PDGF mRNA expression. The results suggest that endogenous PGD2 decreases PDGF synthesis and PDGF mRNA expression, probably through an intracrine mechanism.

W-PO30018

NUCLEAR TRANSLOCATION OF TRANSCRIPTION FACTORS REGULATING INFLAMMATION AND SCLEROSIS IN CIRCULATING LYMPHOMONOCYTES OF PATIENTS WITH IGA NEPHROPATHY

ALESSANDRO AMORE[1], MONICA CHIESA[1], ALESSANDRA MOLLO[1], STEFANIA TURCATO[1], LAURA GHISELLINI[1], PAOLA CIRINA[1], GIOVANNI CONTI[1], LUCA ROASIO[1], LICIA PERUZZI[1], ROSANNA COPPO[1]
Nephrology Dialysis Transplantation Dept, R Margherita University Hospital Torino, Italy[1]

Recent data suggest that gene expression profiles of circulating leukocytes can be a functional fingerprint of IgA nephropathy (IgAN) in phase of clinical activity, confirming 20-year old reports of lymphomonocyte (PBMC) activation observed in IgAN patients. We investigated in PBMC of IgAN patients the intracellular signalling and nuclear translocation of some transcription factors relevant to inflammation/sclerosis.
PBMC from 15 IgAN patients and 15 from healthy controls were tested in unstimulated conditions and after stimulation with Ca++ ionophore and phorbole myristate.
The nuclear binding activity of the following transcription factors, p65 and p50 of the Nuclear Factor kB (NF-kB), Activation Protein (AP-1) and Substance P (SP-1), was evaluated by means of EMSA by using p32 radiolabeled specific probes. Moreover, mRNAs specific for IkB, 2 proteasome and ubiquitin were quantified (relative unit to the housekeeping GAPDH).

Table reports data of autoradiography densitometric analysis of NF-kB binding activity, AP-1 and SP-1 and mRNA expressions in PBMC from healthy controls and IgAN.

	P50 NFkB	P65 NFkB	IkB	2 proteasome	ubiquitin	AP-1	SP-1
Non stimulated controls	104 + 25	50 + 25	0.15 + 0.01	0.18 + 0.01	0.39 + 0.11	30 + 1	15 + 1
Non stimulated IgAN	180 + 11*	267 + 35*	0.45 + 0.02*	0.97 + 0.02*	0.33 + 0.01	300 + 2*	150 + 5*
Stimulated controls	120 + 10	35 + 25				135 + 3	50 + 4
Stimulated IgAN	273 + 9*	296 + 38*				382 + 2*	149 + 5*

Values in IgAN significantly different *$p < 0.01$ vs controls.
Of interest, some correlations with phases of clinical activity were detected.
Our results indicate that PBMC of IgAN patients show the nuclear translocation of transcription factors which are involved in inflammation, proliferation and sclerosis. This observation is extremely interesting, indicating a possible new role of circulating immunocompetent cells in IgAN.

W-PO30019

REACTIVE OXYGEN SPECIES-MEDIATED SIGNALING PATHWAYS IN ANGIOTENSINII-INDUCED MCP-1 EXPRESSION OF PROXIMAL TUBULAR CELLS

CHIAKI TANIFUJI[1], YUSUKE SUZUKI[1], WONG MU GEOT[2], SATOSHI HORIKOSHI[1], TAKESHI SUGAYA[1], MARTA RUIZ-ORTEGA[3], JESUS EGIDO[3], YASUHIKO TOMINO[1]
Juntendo University School of Medicine, Tokyo, Japan[1], University Malaya Medical Center, Kuala Lumpur, Malaysia[2], Autonoma University, Madrid, Spain[3]

Background: Angiotensin II (AngII) has pleiotropic effects, the most well known of which are the generation of reactive oxygen species (ROS) and chemokines in inflammatory lesions. MCP-1 is considered as a major chemokine in the pathogenesis of kidney diseases. Therefore, we examined signaling pathways of AngII-induced MCP-1 expression and the role of ROS in the murine proximal tubular cells (mProx) using various inhibitors. Furthermore, we compared the signaling pathways between mProx and mesangial cells (MC).
Methods: Cells were pretreated with NAC (free radical scavenger; 5 mg/ml), AFC (Ras inhibitor; 12.5 mg/ml), PD98059 (ERK1/2 inhibitor; 25 mM), SB203580 (p38MAPK inhibitor; 10 mM), parthenolide (NF-kB inhibitor; 10 mg/ml), calphostinC (PKC inhibitor; 100 nM), AT1 (ARB) (1 mM) and AT2 receptor antagonist (PD123319; 10 mM). After the pretreatment, cells were exposed to AngII (100 nM) for 6 hours. We examined the MCP-1 expression in mRNA and protein levels.
Results: AngII-induced MCP-1 protein expression in mProx at 6 hours was largely blocked by ROS (NAC; 82 ± 14.0%), Ras (AFC; 82 ± 13.0%) and NF-κB (parthenolide; 89 ± 7.9%) inhibitors. Each of the AT_1 receptor (AT1R) (olmesartan; 41 ± 12.2%) and the AT2R (PD123319; 24 ± 11.2%) antagonists partially blocked the MCP-1 expression. In addition, MAPK pathways in mProx were also involved (PD98059; 57 ± 10.3%, SB203580 32 ± 12.0%) in this protein expression, but it is less-dependent on ROS/Ras pathways. In MC, PKC (calphostin C; 84 ± 2.8%) and NF-κB (89 ± 1.4%) inhibitors strongly attenuated acute AngII-induced MCP-1 expression than ROS/Ras inhibitors (1.0 ± 0.9/29 ± 9.5%). MAPK pathways are involved in MCP-1 expression (PD098059; 54 ± 3.5%). Especially, p38 MAPK (SB203580; 57 ± 8.5%) inhibitor blocked in MC more than in mProx. AT1R (69 ± 8.6%) and AT2R (57 ± 21.0%) antagonists also blocked in MC.
Conclusion: These findings indicate that, although NF-κB activation is critical for AngII-induced MCP-1 expression, signaling pathways involved are different in the both renal resident cells. ROS-mediated signalings in mProx may contribute to AngII-induced inflammatory responses much more than to those in MC.

W-PO30020

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORγ AGONISTS INHIBIT TGF-β1-INDUCED FIBROTIC RESPONSES ON RAT RENAL INTERSTITIAL FIBROBLASTS

FENG LIU[1], WANG WEIMING[1], XING JINGPING[1], CHEN NAN[1]
Department of Nephrology, Ruijin Hospital, Shanghai Second Medical University, Shanghai, 200025, P.R. China[1]

TGF-β1) plays a key role in renal interstitial fibrosis. Connective tissue growth factor(CTGF) is a key factor regulating TGF-β1-induced extracellar matrix(ECM) production and inhibiting TGF-β/Smad signal pathway may provide a novel approach to controlling renal interstitial fibrosis. Peroxisome proliferator-activated receptor-γ(PPARγ) agonists have a potential role in ameliorating inflammation and growth of cancer cells. The aim of our study is to investigate the effects of its agonists on TGF-β1-indued fibrotic responses.
Methods: In NRK/49F cells, TGF-β1-indued CTGF fibronectin(FN) and type III collagen(Col III) production were detected by RT-PCR and Western blot. TGF-β1-induced Smad2, Smad3 and phosphorated-Smad2/3(p-Smad2/3) protein expression were examined by Western blot analyses.
Results: In NRK/49F cell, a basal level of CTGF, FN and Col III mRNA expression were detected, TGF-β1 enhanced their expression in a dose- and time-dependent manner, with the maximal response at a concentration of 5 ng/ml and the best stimulating time course at 24 hour. The level of FN protein expression had the same appearance. A basal level of Smad2, Smad3 and p-Smad2/3 protein expression were detected by Western blot analyses. However, p-Smad2/3, other than Smad2 and Smad3 protein expression in NRK/49F cells began to increase at 15 minutes and reached its peak at 2 hours after treatment with 5 ng/ml TGF-β1. When NRK/49F cells were pretreated by PPAR-γagonists, 15d-PGJ2 troglitzone and ciglitazone for 2 hours, the level of CTGF, FN and Col III expression induced by TGF-β1 decreased. The level of p-Smad2/3 protein expression induced by TGF-β1 were downregulated by 15d-PGJ2 troglitazone and ciglitazone.
Conclusion: PPAR-γagonists have a potential role in inhibiting the fibrotic responses induced by TGF-β1, possibly by blocking the phosphoration of Smad2/3 induced by TGF-β1.These findings may providea therapeutic implication of PPAR-γ agonists in the prevention and intervention in renal interstitial fibrosis.

W-PO30021

A NEW STRATEGY TO TREAT RENAL FIBROSIS: TARGETING OF P38 MAPK INHIBITOR SB202190 TO THE KIDNEY

JAI PRAKASH[1], KLAAS POELSTRA[2], FRITS MOOLENAAR[2], ROBBERT JAN KOK[2]
Groningen University Institute for Drug Exploration (GUIDE)[1], Department of Pharmacokinetics and Drug Delivery, Groningen University Institute for Drug Exploration (GUIDE), Netherlands[2]

P38 mitogen-activated protein kinase (MAPK) plays a pivotal role in signaling cascades in inflammation and fibrosis. We hypothesized that blockade of p38-MAPK locally within the kidneys might be beneficial in inhibiting tubulo-interstitial fibrosis. In the present study, we explored the effect of the p38-MAPK inhibitor SB202190 on fibrotic signaling in renal tubular cells (NRK-52E). Since the administration of a p38-MAPK inhibitor will cause systemic side-effects, renal-specific delivery may be a relevant approach to treat renal fibrosis. We therefore developed a novel renal-specific drug targeting construct of SB201290 and the carrier protein lysozyme.
Results: NRK-52E cells were incubated for 24 hours with bovine serum albumin (BSA, 30 mg/ml) to mimic proteinuria-induced inflammation or with TGF-b1 (10 ng/ml). The m-RNA expressions of MCP-1, TIMP-1, procollagen-1A1 and a-SMA were examined using real-time RT-PCR. Pretreatment with SB202190 (10 μM) inhibited BSA-induced MCP-1 expression significantly and also reduced the expression of TIMP-1 and procollagen-1A1 after BSA or TGF-b1 treatment.
For renal-selective delivery of SB202190, we conjugated the drug covalently to lysozyme at a 1 : 1 molar ratio. The developed drug-lysozyme conjugates were characterized using mass-analysis and by HPLC determination of the drug after degradation of the conjugate. In vitro drug-release studies with kidney homogenate at 37°C showed that SB202190 was released slowly from the conjugates during 24 h, while it remained stable in serum. In the past, we have already demonstrated that lysozyme and its conjugates are efficiently accumulated in the proximal tubules of the kidneys after in vivo administration. Based on

these studies, we will now test the SB202190-lysozyme conjugates in animal studies for renal fibrosis.
In conclusion, the present study suggests that SB202190 has significant antifibrotic effects in-vitro in renal tubular cells and that renal-specific delivery of SB202190 can be achieved. Renal delivery of p38 MAPK inhibitor may be a promising new approach to treat renal fibrosis.

W-PO30022

CALCYCLIN AND ANNEXIN A2 ARE RAPID RESPONSE GENES TO SENSING TUBULAR INJURY AND RECOVERY IN ACUTE RENAL FAILURE

CHAO-WEN CHENG[1], SHUK-MAN KA[1], ZHI-XIANG FAN[2], WEI-HWA LEE[3], ANN CHEN[3]
Graduate Institute of Life Sciences, National Defense Medical Center, Taiwan[1], Graduate Instiute of Pathology, National Defense Medical Center, Taiwan[2], Departmant of Pathology, National Defense Medical Center, Taiwan[3]

Acute tubular necrosis (ATN) is the most common pathologic entity responsible for the clinical state of acute renal failure (ARF). Quiescent cells in the renal tubule would normally divide and differentiate during ATN via as yet unclear mechanism to restore the functional integrity of the nephron.
In a mouse model of toxic nephropathy induced by the administration of uranyl nitrate, we observed the development of ATN 3–7 days after induction, followed by regeneration of renal tubular epithelial cells approximately 2 weeks after induction, which was characterized by increased dedifferentiated and mitotic cells in the damaged tubules. Real-time PCR analysis of mRNA expression of a large family of intracellular calcium and phospholipid-binding proteins demonstrated selective expression of Calcyclin (*S100A6*) and *Annexin A2* (*Anxa2*) in the renal cortex was greatly elevated on day 3, and gradually declined on day 7 and was only slightly elevated on day 14. Similarly, the expression of both proteins, as demonstrated by immunohistochemistry (IHC) and Western blot analysis, was increased and reached the peak level on day 7 and then gradually declined by day 14. As demonstrated by IHC, vimentin, a marker of dedifferentiated cells, was highly expressed during the recovery phase. By combined *in situ* hybridization and IHC, co-localization of S100A6 & proliferating cell nuclear antigen (PCNA), and Anxa2 & PCNA was identified, respectively, indicating that most PCNA-positive tubular cells expressed both S100A6 and Anxa2. The universality of this phenomenon was confirmed in two other mouse ATN models, the ischemic-reperfusion injury and folic acid-induced ARF. The pattern of mRNA expression of the two genes was very rapid and similar in pattern to that of ATN induced by uranyl nitrate.
Collectively, these findings demonstrate an important role for S100A6 and Anxa2 in sensing injury and recovery of tubular cells by regeneration in acute renal failure.

W-PO30023

CONSTRUCT OF ANTISENSE LCK RNA RECOMBINATE ADENOVIRUS VECTOR AND INVESTIGATION OF ITS EFFECT ON TUBULAR EPITHELIAL CELLS

QING GANG LI[1], YING HUI DENG[1], ZHI GANG WANG[1], WENHU LIUA[1], XIUHONG XUB[2], CHONGYU RENC[3]
Department Of Nephrology, Beijing Friendship Hospital, China[1], Department Of Urology, Beijing Friendship Hospital Affiliating Capital University Of Medical Sciences, Beijing, China[2], Department of Pathophysiology, Peking Union Medical College, Beijing, China[3]

Lymphocyte-specific protein-tyrosine kinase (p56 lck) has been implicated as an important tyrosine kinase, which was located in T cells and also found in tubular epithelial cells recently, and takes part in renal injury through IL-12 or Il-2. Both IL-12 and IL-2 activate a wide variety of cellular responses, including lck, c-jun, and apoptosis. If we block expression of Lck, what its effect on tubular cells is not known. In the present study we constructed the antisense lck recombinate adenovirus vector and investigated the role of its effect on tyrosine kinase lck expression in tubular epithelials. Antisense Lck RNA was constructed by adenovirus vector, which was expressed in tubular cells. Treatment with antisense lck suppressed c-jun gene expression in a time-dependent manner in tubular cells, suggesting the critical role of lck kinase. lck was also found to be required for IL-12-induced c-jun activation in tubular cells. Overall our results demonstrate that lck kinase plays a critical role in the activation of c-jun, and apoptosis. When we blocked the expression of lck in tubular epithelial cells by antisense lck RNA, cell apoptosis was decreased.

Lipid Mediators/Eicosanoids

W-PO30024

LOCALIZATION OF CYP4A AND CYP4F SUBFAMILIES IN HUMAN KIDNEY

OSAMU ITO[1], YASUHIRO NAKAMURA[1], LIPING TAN[1], YUKO SASAKI[1], NAOYOSHI MINAMI[1], MASAYUKI KANAZAWA[1], SADAYOSHI ITO[1], HIRONOBU SASANO[1], MASAHIRO KOHZUKI[1]
Tohoku University Graduate School of Medicine[1]

Members of the cytochrome P-450 (CYP) 4A and CYP4F subfamilies catalyze the omega-hydroxylation of fatty acids. 20-hydroxyeicosatetraenoic acid (20-HETE), the omega-hydroxylated metabolite of arachidonic acid, is a natriuretic and vasoactive substance and has been shown to regulate tubular ion transport, vascular tone, glomerular protein permeability and blood pressure in animal models. The present study determined the localization of CYP4A and CYP4F subfamilies in human kidney. The specificity of polyclonal antibodies raised against human CYP4A11 and CYP4F2 was tested by immunoblots of recombinant human CYP4 proteins from Sf9 insect sells. The polyclonal CYP4A11 antibody reacted with the CYP4A11 protein, but not with the CYP4F2, CYP4F3A, CYP4F3B or CYP4F12 protein. The polyclonal CYP4F2 antibody reacted with the CYP4F2, CYP4F3A, CYP4F3B and CYP4F12 proteins, but not with the CYP4A11 protein. Immunohistochemical experiments with the polyclonal CYP4A11 and CYP4F2 antibodies revealed the localization of CYP4A and 4F proteins in human kidney. In the renal cortex, strong stainings for CYP4A11 were detected in the proximal tubules, and lower stainings were detected in the distal tubules, but staining was not detected in the renal vasculature or glomeruli. In the medulla, stainings for CYP4A11 were detected in the thick ascending limbs (TAL) and the collecting ducts. Stainings for CYP4F2 were observed generally in the proximal tubules, TAL, and collecting ducts, but not in the renal vasculature or glomeruli. These results indicate that CYP4A and CYP4F subfamilies are widely expressed in the renal tubules of human, and 20-HETE may regulate the tubular functions and salt-sensitivity in blood pressure. The contribution of 20-HETE to the control of renal vascular tone or glomerular protein permeability is probably little, because neither CYP4A nor CYP4F is expressed in the renal vasculature or glomeruli.

W-PO30025

LXA4 INHIBITS TNF-A-INDUCED PRODUCTION OF INTERLEUKINS AND PROLIFERATION OF MESANGIAL CELLS

SHENG-HUA WU[1], CHAO LU[1], LING DONG[1], GUO-PING ZHOU[1]
Department of Pediatrics, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China[1]

To examine whether LXA4 had an inhibitory effects on TNF-α-induced productions of IL-1β and IL-6 and proliferation of glomerular mesangial cells of rat, cultured mesangial cells were treated with TNF-α (10 ng/mL), with or without preincubation with LXA4 at the different concentrations. Cell proliferation was assessed by [3H]-thymidine incorporation. IL-1β and IL-6 in supernatant were analyzed by ELISA. Expressions of IL-1β and IL-6 mRNA were determined by real-time PCR and cyclin E by RT-PCR. Proteins of cyclin E, threonine phosphorylated Akt1 (Thr308) and p27kip1 were analyzed by Western blotting. Activities of STAT3, NF-κB were determined by EMSA. Expression of Src homology 2-containing protein-tyrosine phosphatase (SHP-2) was assessed by immunoprecipitation and immunoblotting. TNF-α-stimulated proliferation, releases of proteins and expressions of mRNA of IL-1β and IL-6 in mesangial cells were inhibited by LXA4. The marked increments in mRNA expression and protein synthesis of cyclin E induced by TNF-α were down-regulated by LXA4. LXA4 antagonized the phosphorylation of SHP-2 and Akt1 (Thr308), and activities of NF-κB and STAT3 induced by TNF-α. Pretreatment of the cells with NF-κB inhibitor PDTC blocked the productions of IL-1β, IL-6 and activation of NF-κB induced by TNF-α. TNF-α-induced decrement in expressions of p27kip1 protein was ameliorated by LXA4 in a dose-dependent manner. In conclusion, TNF-α-induced proliferation and increment of cyclin E of rat mesangial cells can be inhibited by LXA4, and these inhibitory effects might be through the mechanisms of STAT3 and Akt1/p27kip1 pathway-dependent signal transduction. LXA4 also antagonized TNF-α-stimulated IL-1β and IL-6 synthesis, and these antagonisms were related to SHP-2 and NF-κB pathway-dependent signal transduction.

W-PO30026

THE CHARACTERISTICS OF LOW-DENSITY LIPOPROTEIN (LDL) PARTICLE SIZE IN HYPERCHOLESTEROLEMIC PATIENTS WITH CHRONIC GLOMERULONEPHRITIS

GEN YASUDA[1], SHIN-ICHIRO MASUDA[1], NOBUHITO HIRAWA[1], DAISAKU ANDO[1], TERUHIKO ENDO[1], KYOKO DEJIMA[1], SATOSHI UMEMURA[1]
Yokohama City University Center Hospital[1]

Purpose of the study: Small dense LDL (sd-LDL) is more atherogenic than the large LDL, playing an important role in an increased risk of coronary heart disease. However, there are few reports on the characteristics of sd-LDL in hypercholesterolemic patients with chronic glomerulonephritis (CGN) and moderate renal impairment. We evaluated the LDL particle size and its relation to other parameters in these patients and compared the results with those obtained from patients with diabetic nephropathy (DN).
Methods: Serum lipid parameter and creatinine concentrations were measured in 28 patients with CGN and 18 patients with DN. LDL particle size was measured by gradient gel electrophoresis.
Results: The ratio between pattern A (LDL particle diameter >25.5 nm) and B (LDL particle diameter <25.5 nm) was 16/12 in the CGN group, which was not different ($\chi^2 = 1.15$, $P = 0.28$) from the ratio of 8/10 in the DN group. LDL particle size was positively correlated with serum high-density lipoprotein concentrations in the CGN group ($r = 0.56$, $P < 0.01$) and DN group ($r = 0.59$, $P = 0.02$), and inversely correlated with serum triglyceride concentrations in the CGN group ($r = -0.55$, $P = 0.01$) and DN group ($r = -0.60$, $P = 0.01$). However, LDL particle size was only inversely correlated with serum creatinine concentrations in the CGN group ($r = -0.45$, $P = 0.02$) but not in the DN group ($r = -0.32$, $P = 0.23$).
Conclusions: The results suggest that relation between LDL particle size and the other lipid parameters did not differ between CGN and DN groups, however, LDL particle size decreased in line with renal impairment only in the CGN group, indicating that sd-LDL is more prominent when impairment of renal function advances in this group.

W-PO30027

TRANSFECTION OF LXA4 RECEPTOR HOMOLOGUE GENE INTO MESANGIAL CELLS INTENSIFIES THE INHIBITORY EFFECT OF LXA4 ON CTGF-INDUCED CHEMOKINE RELEASE

SHENG-HUA WU[1], CHAO LU[1], LING DONG[1], GUO-PING ZHOU[1]
Department of Pediatrics, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China[1]

To examine whether lipoxin A4 (LXA4) has inhibitory effects on connective tissue growth factor (CTGF)-induced chemokine release by mesangial cells, the cultured mesangial cells of rats were treated with CTGF, with or without preincubation with LXA4. The Proteins of fractalkine, monocyte- chemoattractant protein-1 (MCP-1) and RANTES in the supernatants were determined by ELISA. The chemotactic effects of the supernatants on monocytes was assessed by the in vitro chemotaxis assay. The expressions of mRNA of these chemokines were analyzed by RT-PCR. The expressions of phospho(P)-p42/44 MAPK were assessed by Western blotting. Treatment of the cells with CTGF enhanced the mRNA expressions of fractalkine, MCP-1 and RANTES, the expressions of P-p42/44MAPK and concentrations of above chemokines in the supernatants. Pretreatment of the cells with PD98059, an inhibitor of P-p42/44 MAKP, decreased the CTGF-induced expressions of P-p42/44 MAPK and the levels of above chemokines in supernatants. Pretreatment of the supernatants of CTGF-treated cells with anti-fractalkine, MCP-1 or RANTES antibodies partially inhibited the chemotactic effect of the supernatants on monocytes. Pretreatment of the cells with LXA4 inhibited the CTGF-stimulated mRNA expressions of above chemokines, the expressions of P-p42/44MAPK and the concentrations of above chemokines in the supernatants. To explore whether transfection of LXA4 receptor homologue gene (LRHG) into mesangial cells intensified these inhibitory effects of LXA4, mesangial cells were transfected with pcDNA3.1/LRHG vector and their expressions were detected by Western blotting. The transfection of pcDNA3.1/LRHG vector intensified the inhibitory effects of LXA4 on CTGF-induced mRNA expressions and release of the above chemokines and expression of the P-p42/44 MAPK as compared with vector-transfected mesangial cells.

W-PO30028

OXIDATIVE STRESS IN THE NEPHROTIC SYNDROME

SUBHASH PILLAI[1], SU SAWANT[2], LJ JOSEPH[2], MGR RAJAN[2], AR HALANKAR[1], AF ALMEIDA[1]
Seth GS Medical College and KEM Hospital, Parel, Mumbai[1], Bhabha Atomic Research Centre, India[2]

Altered oxidant–antioxidant status has been established as a risk factor for progressive renal scarring in patients with the nephrotic syndrome [NS]. The lipid peroxidation marker malonyldialdehyde (MDA) and the antioxidant enzymes [catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx)] were measured in the serum of 50 adult patients [age range 16–55 years] with documented history of a NS and without significant renal dysfunction [serum creatinine <1.2 mg%]. Patients with diabetes, active infection, secondary glomerular disease and reduced renal mass were excluded. Out of the 50 patients enrolled, 18 were in complete remission and had not received immunosuppressive agents in the 6 months period prior to enrolment [Group 1]. Sixteen patients with persistent proteinuria (protein excretion <3.5 gm/day/1.73 m^2 BSA) [Group 2] and a further 16 with persistent nephrotic-range proteinuria [Group 3], were also included. Fourteen age-matched subjects formed the controls [Group 0]. MDA was estimated using the thiobarbituric acid assay, SOD by modified Marklund and Marklund, CAT by Aebi's method and GPx by modified Haffeman's method. Statistical analysis was performed using SPSS software.The oxidative stress markers were correlated with the risk factors for disease progression, namely, the degree of proteinuria, hypertension, hyperlipidemia and dietary protein intake. Positive correlations were noted between the degree of proteinuria and serum MDA levels [$r = 0.258$, $p = 0.035$, $n = 50$] and between the total cholesterol and serum MDA levels [$r = 0.212$, $p = 0.046$, $n = 50$] and a significant negative correlation between the degree of proteinuria and the antioxidant activity of catalase [$r = 0.245$, $p = 0.043$, $n = 50$]. However, comparisons among the proteinuric groups and also with the controls, did not show any statistically significant differences in the oxidative stress markers probably due to small sample sizes.
In conclusion, proteinuria and hypercholesterolemia showed statistically significant correlation with oxidative stress in NS.

W-PO30029

GENE EXPRESSION PROFILE OF ADIPOCYTOKINES IN ALDOSTERONE-INDUCED HYPERTENSIVE RENAL INJURY RATS

MIKI NAGASE[1], SHIGERU SHIBATA[1], MASAYO HINO[1], MASAKO YAGO[1], SHINYA KANAME[1], TAKANARI GOTODA[1], TOSHIRO FUJITA[1]
Division of Nephrology and Endocrinology, University of Tokyo Graduate School of Medicine[1]

Background: It has been proposed that adipocytokines derived from adipose tissues play an important role in the pathogenesis and prognosis of cardiovascular and renal diseases. The purpose of the present study is to investigate the expression and modulation of adipocytokines in the visceral and subcutaneous adipose tissues of aldosterone-induced hypertensive renal injury model.
Methods: Male Sprague-Dawley rats were uninephrectomized, infused with aldosterone via osmotic minipump and fed a 8% NaCl diet for 4 weeks. Total RNA was extracted from subcutaneous and visceral (mesenteric, retroperitoneal and epididymal) adipose tissues. Gene expression was quantitatively analyzed using real time RT-PCR. Isolated adipocytes were prepared from the above-mentioned adipose depots of normal rats by collagenase treatment according to Rodbell's method.
Results: Aldosterone-infused rats on high salt diet developed severe hypertension and massive proteinuria. Serum leptin concentration was significantly reduced in aldosterone-administered rats as compared with controls. On the other hand, serum adiponectin level was slightly increased in aldosterone group. Analysis of adipose depot-specific distribution revealed that leptin, adiponectin and angiotensinogen were expressed predominantly in visceral adipose tissues compared with subcutaneous fat tissue in control rats. In aldosterone-treated rats, mRNA expression of leptin was dramatically attenuated in various adipose tissues. On the other hand, expressions of adiponectin and angiotensinogen were enhanced in retroperitoneal and epididymal adipose tissues of aldosterone group. The transcripts for leptin, adiponectin and angiotensinogen were also detected in isolated adipocytes.
Conclusion: Our data indicated that leptin expression was reduced whereas adiponectin and angiotensinogen expressions were enhanced in the visceral adipose tissues of aldosterone-infused hypertensive renal injury rats. Alteration

of these fat-derived adipokines, for example augmented angiotensinogen production, might contribute to the progression of renal dysfunction in this model.

Apoptosis in Inflammation

W-PO30030

CHARACTERISATION OF A CELL DEATH GENE, DWNN, IN HIVAN

SARALADEVI NAICKER[1], ZUKILE MBITA[1], ZODWA DLAMINI[1], STEWART GOETSCH[1], JASPER REES[1]

University of the Witwatersrand, Johannesburg, South Africa[1]

Infection of T-cells by HIV leads to their destruction through apoptosis. HIV infection leads to various renal complications, which include HIV–associated focal segmental glomerulosclerosis and related mesangiopathies. HIVAN is viewed as the result of insufficient renal cell turnover due to HIV infection, due to apoptosis. DWNN (domain with no name) is a novel gene that is expressed into DWNN domain, a 76-residue domain that is conserved across organisms. Knockouts of the gene were found to be resistant to CTL killing and apoptosis induced by a chemical inducer staurosporine.

Aim: The aim of this study was to characterize the DWNN gene in HIVAN. The expression levels were correlated with apoptosis levels.

Methods: RNA extracted from the kidney cell line Graham 297 was used in RT-PCR to investigate the number of DWNN transcripts. Renal biopsy sections were processed for immunohistochemistry to localize both the DWNN RNA and protein. DWNN RNA localization was correlated to p53 localization. TUNEL was used to detect apoptosis.

Results: Reverse transcription PCR shows that in the normal kidney, exon 16 of DWNN gene is alternatively spliced. In situ hybridization studies revealed high levels of DWNN mRNA in the interstitial tissue of these patients and this correlated with increased apoptosis levels in this region. In the glomerulus, DWNN is highly expressed at both mRNA and protein levels. Immunocytochemistry shows that the DWNN protein is up-regulated as compared to the normal kidney. These results also correlated with p53 localization in all the HIVAN biopsies investigated.

Conclusion: This study demonstrates that DWNN is involved in apoptosis in the p53-dependent apoptosis pathway in HIVAN.

W-PO30031

APOPTOSIS IN EXPERIMENTAL DIABETIC NEPHROPATHY

ENYIOMA N. OBINECHE[1], ABDU ADEM[2], SI CHANDRANATH[2], AM SALEH[3], C VIJAYASARATHY[3], S GALADARI[3], M PATEL[3], I AHMED[4]

Internal Medicine, Faculty of Medicine & Health Sciences, UAE University[1], *Department of Pharmacology*[2], *Department of Biochemistry*[3], *Department of Anatomy*[4]

The aim of this study was to investigate the morphological and biochemical apoptotic changes that occur at 1 and 8 months after induction of diabetes by streptozotocin. We also investigated the specific role of apoptosis-regulatory genes in diabetic nephropathy.

Diabetes was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg body weight). Tissues from diabetic ($n = 15$ each) and control ($n = 15$ each) rats were analyzed after 1 and 8 months of treatment. Electron microscopy showed basement membrane thickening, loss of podocytic foot processes, disruption of tubular basal infoldings and their related mitochondria 8 months after induction of diabetes. Biochemical results showed activation of the effector caspase, caspase-3 and cleavage of its natural substrate PARP, a DNA repair enzyme, indicating activation of apoptotic cascades in diabetic kidney cells. Furthermore, cytochrome-c released from mitochondria of diabetic kidney cells into the cytoplasm, indicating disruption of mitochondrial outer membrane, strongly suggests involvement of cytochrome-c mediated activation of apoptotic pathway in these cells. Interestingly, the level of expression of p53 was substantially increased in diabetic kidney cell lysates. p53 is known to induce cytochrome-c mediated activation of caspase-3. We also observed changes in the level of anti-apoptotic protein Bcl-2 in diabetic kidney mitochondrial fractions. The electron microscopic results suggest that extensive damage and loss of kidney tissue might be partly responsible for the clinical presentation of diabetic nephropathy. The ultrastructural changes in the tubules seem to implicate apoptosis in the pathology of diabetic nephropathy. Moreover, the observed increase in cytosolic cytochrome-c, activation of caspase-3, cleavage of PARP and changes in the expression levels of Bcl-2 and p53 in diabetic kidney cells indicate involvement of a mitochondrial pathway in triggering apoptosis in diabetic nephropathy.

W-PO30032

MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1) INHIBITS FAS-INDUCED APOPTOSIS AND INDUCES ALPHA-SMOOTH MUSCLE ACTIN NEOEXPRESSION IN EPITHELIAL TUBULAR CELLS

TERESA RAMPINO[1], MARILENA GREGORINI[2], RENZA TIBOLDO[3], ELISABETTA ROSCINI[2], CRISTINA GUIDETTI[3], VITTORIO NECCHI[2], MILENA MAGGIO[3], ALESSANDRA MASSOGLIA[2], CHIARA BALENZANO[3], GIOVANNI PIOTTI[4], SIMONA SITA[2], ILARIA SERRA[3], GIULIA BEDINO[2], ANTONIO DAL CANTON[3]

Unit of Nephrology, Dialysis and Transplantation and University of Pavia[1], *Unit of Nephrology*[2], *Dialysis and Transplantation IRCCS Policlinico San Matteo*[3], *Unit of Pathology IRCCS Policlinico San Matteo*[4]

MCP-1 is a chemokine that recruits leukocytes to inflammatory tissues and induces interstitial fibrosis in chronic renal disease. Until now we knew that the receptor of MCP-1, CCR2 was expressed exclusively in inflammatory cells. In this study we investigated CCR2 expression in the kidney in vivo and in proximal tubular cells (HK2) in vitro. In addition, we studied whether MCP-1 induces in HK2 cells biological effects that may modulate interstitial fibrosis. CCR2 was evaluated by immunohistochemistry and immunogold electronic microscopy in normal renal tissue, and by flow cytometry in HK2 cells incubated for 24 h in the presence and absence of MCP-1 (10 ng/ml). In order to study the effects of MCP-1 on TNF-alpha-induced apoptosis, HK2 cells (5×103) were prestimulated with gamma-IFN (24 h) to induce Fas/CD95 surface expression; then part of the cells were conditioned with TNF-alpha, part with MCP-1 (10 ng/ml) followed by TNF-alpha. After treatment the cells were fixed and stained with nuclear dye bisbenzimide, and the percentage of apoptotic cells was determinated counting nuclei with condensed or fragmented chromatin at immunofluorescence microscopy. The effect of MCP-1 on epithelial to mesenchymal transition was investigated by studying the expression of alpha smooth-muscle actin in HK2 cells stimulated with MCP-1 (10 ng/ml). The results in vivo show that renal tubules express CCR2, and the receptor is localized on mitochondria and on plasma membrane. In vitro, MCP-1 increases CCR2 expression (CCR2-positive cells: basal 7.0%, stimulated with MCP-1 16.9%, $p < 0.001$), inhibits Fas-induced apoptosis (basal 16.0%, stimulated 6.1%, $p < 0.05$) and induces neoexpression of alpha smooth-muscle actin. The expression of CCR2 in tubular cells in vivo and in vitro and the biological effects of MCP-1 on tubular cells in vitro indicate that tubular cells are a target of MCP-1. The direct effects of MCP-1 on tubular cells may play a relevant role in modulating interstitial fibrogenesis.

W-PO30033

THE EFFECTS OF ATORVASTATIN ON APOPTOSIS AND INFLAMMATION FOLLOWING ISCHAEMIA/REPERFUSION (I/R) IN THE KIDNEY

BIN YANG[1], JOHN HAYLOR[2], KEVIN HARRIS[1], ALAN BEVINGTON[1]

John Walls Renal Unit, Leicester General Hospital, University of Leicester, Leicester[1], *Sheffield Kidney Institute, Northern General Hospital, University of Sheffield, Sheffield*[2]

HMG-CoA reductase inhibitors may have beneficial effects on renal injury independent of lowing lipid effects, although the underlying mechanism is not defined. We examined markers of apoptosis and inflammation in a rat model of ischaemia (45 mins) and reperfusion (4, 24, 48 and 120 hrs) with uninephrectomy and the effects of 10 mg/kg atorvastatin (AT) administered intravenously after clamping the renal artery in the group with 24 hrs of reperfusion. Caspase-3 activity was increased with the time of reperfusion (0.003 ± 0.0005; 0.004 ± 0.0008; 0.005 ± 0.0004 and 0.005 ± 0.009 pmol AMC liberated/min/mg protein) and AT increased this to 0.005 ± 0.0008 at 24 hrs. Apoptotic cells (per 400′ field, TUNEL assay) were gradually increased in tubular areas (0.444 ± 0.100; 3.694 ± 2.894; 6.389 ± 3.229 and 3.667 ± 3.296); interstitial apoptosis was significantly higher at 4 hrs (2.944 ± 0.447) compared with other time points (0.278 ± 0.056; 0.167 ± 0.048 and 0.556 ± 0.274). AT doubled tubular apoptosis (7.833 ± 0.441), but had little effect on interstitial apoptosis (0.139 ± 0.073). Inflammatory cells (ED1 staining, per 400′ field of tubulointerstitium) were 0.681 ± 0.132 at 4 hrs, dropped significantly at 24 hrs (0.139 ± 0.050), but then progressively increased again (0.431 ± 0.169 at 48 hrs, and 0.958 ± 0.461 at 120 hrs, $p < 0.05$ compared with all other time points). At 24 hrs AT (0.208 ± 0.024) had no significant effect on ED1 staining. Serum creatinine rose following I/R (121 ± 10; 255 ± 81; 292 ± 179 and 322 ± 205 mmol/l) and was not affected by AT (252 ± 68). The effects of AT on caspase-3 was also examined in both cultured normal rat proximal tubular cells and LLC-PK1 cells. Over the dose range 0.75 to 400 mM AT, a biphasic dose response was observed on caspase-3 activity with a gradual increase at low doses (up to 50 mM) but decrease at higher doses. In conclusion I/R injury results in apoptosis and inflammation, both of which are key features of the renal injury. AT may modulate these changes. The long-term effects of AT on renal recovery following I/R remain to be determined.

W-PO30034

EFFECT OF WAVES LITHOTRIPSY IN IMMORTALIZED HUMAN MESANGIAL CELLS (IHMC)

ANDREIA SILVA DE OLIVEIRA[1],
CARLOS EDUARDO CARVALHO DE MATOS[1],
CLARA VERSOLATO RAZVICKAS[1], OTONIEL RIBAS[1],
RONALDO SOARES MAIA[1],
VICENTE DE PAULO CASTRO TEIXEIRA[1], NESTOR SCHOR[1],
SANDRA MARIA RODRIGUES LARANJA[1]
Escola Paulista de Medicina – UNIFESP[1]

Introduction and objectives: The renal stones treatment with extracorporeal shock waves lithotripsy (SWL) is widely used although some aspects of the effects of this procedure on the different renal cells have not been evaluated. The aim of this study is to evaluate the effects of SWL in Immortalized Human Mesangial Cells (IHMC) in culture submitted to different numbers of SWL, side effects or differences in the parameters analysed.
Methods: IHMC were submitted to 1000 and 4000 SW with 17 Kv, 90 Mhz of intensity. Intracellular calcium $[Ca^{+2}]_i$ was analysed by spectrofluorimetry with the intracellular probe FURA – 2. Cell viability was analysed by acridine orange and ethyde bromide method and apoptosis by Hoescht 33342 method. The preliminary results are showed in the table above.
Results: $[Ca^{+2}]_i$ is presented in values ratio (340/380), viability and apoptosis are presented in percentage.

	$[Ca^{+2}]_i$	after All	viability (%)	apoptosis (%)
Control (C)	1.81 ± 0.06 n = 26	2.18 ± 0.08 n = 26	72.25 ± 4.75 n = 4	12 ± 0.57 n = 4
1000 SW	2.30 ± 0.39* n = 16	2.74 ± 0.41* n = 16	76.30 ± 1.30 n = 14	31.14 ± 1.36 n = 14
4000 SW	1.66 ± 0.21 n = 8	2.00 ± 0.32 n = 8	77.33 ± 1.38 n = 6	25.84 ± 2.32 n = 6

Conclusions: There were no significant differences in the viability and apoptosis in the cells treated with 1000 and 4000 SW. However there was a significant increase of 27% in the $[Ca^{+2}]_i$ after 1000 SW (*20% or more are significant) suggesting cell injury. Surprising in the cells treated with 4000 SW this increase did not occur, its possible that there was a calcium leak or a up regulation of the organelas responsible by the intracellular control happened but more experiments are necessary to confirm and elucidate this fact.

W-PO30035

PANCASPASE INHIBITION IS PROTECTIVE AGAINST CISPLATIN-INDUCED ACUTE RENAL FAILURE (ARF)

SARAH FAUBEL[1], LEONID REZNIKOV[1], HILARY SOMERSET[1],
BELDA DURSON[1], CHARLES A DINARELLO[1], CHARLES L EDELSTEIN[1]
Internal Medicine, University of Colorado Health Sciences, United States[1]

We have demonstrated that caspase-1 deficient mice are functionally protected against cisplatin-induced ARF (Kidney International, 2004). Protection was associated with decreased renal neutrophil accumulation and apoptosis. Caspase-1 activates the proinflammatory cytokines IL-1β and IL-18. Caspase-3 is pro-apoptotic. Therefore, we sought to examine the role of IL-1β, IL-18 and caspase-3 in cisplatin-induced ARF. Cisplatin (30 mg/kg) was injected IP into C57BL/6 mice. Renal dysfunction occurred on day 3: serum creatinine (Cr)(mg/dl) was 0.2 in vehicle–treated (Veh) vs. 2.1 in cisplatin-treated (Cis). Renal IL-1β and IL-18 (pg/mg) were determined on days 1, 2 and 3 post-injection by electrochemiluminescence. On day 1, IL-1β and IL-18 were unchanged. IL-1β was 2.4 in Veh vs. 3.4 in Cis ($P < 0.01$) on day 2, and 1.8 in Veh vs. 3.2 in Cis ($P < 0.001$) on day 3. IL-18 was 13 in Veh vs. 32 in Cis ($P < 0.001$) on day 2, and 9 in Veh vs. 25 in Cis ($P < 0.001$) on day 3. IL-1 receptor antagonist (IL-1Ra) (30 mg/kg, 3 times daily) or vehicle (V) was administered IP beginning at the time of cisplatin injection. Cr was 1.1 in V and 1.1 in IL-1Ra (P = NS, N = 10). 300µL of IL-18 antiserum (AS) or V was administered IP two hours prior to cisplatin injection. Cr was 2.2 in V and 2.7 in AS (P = NS, n = 9). 2 mg twice a day of the pan-caspase inhibitor IDN-8050 (IDUN pharmaceuticals) or vehicle (DMSO) was administered beginning 3 hours after cisplatin injection. IDN-8050 inhibits both caspase-1 and caspase-3. Cr was 2.5 in vehicle-treated and 1.6 in IDN8050-treated ($P < 0.05$, n = 5–7). In summary, IL-1β and IL-18 increase prior to renal dysfunction in cisplatin-induced ARF. However, neither IL-1Ra nor IL-18 antiserum attenuates ARF whereas pan-caspase inhibition with IDN-8050 is protective. In conclusion, the potential therapeutic role of pan-caspase inhibition in cisplatin-induced ARF merits further study.

Basic Immunology and Inflammation

W-PO30037

REDUCTION OF CUBILIN MRNA BY RNAI RESULTS IN INHIBITION OF ALBUMIN-INDUCED MCP-1, RANTES EXPRESSION BY RENAL TUBULAR EPITHELIAL CELLS

YN HE[1], YANG JR[1], LIN JING[1], LIANG HAIJUN[1], JIANG JX[1], LI XINLU[1]
Dept. of Nephrology, Daping Hospital of TMMU, China[1]

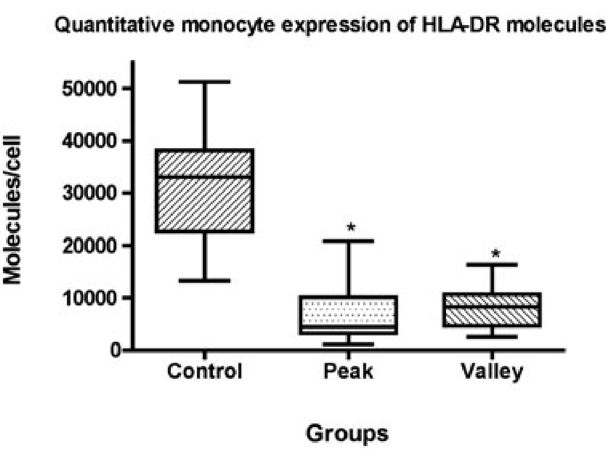
Background: Proteinuria has been demonstrated to be responsible for the progress of chronic kidney diseases. Albumin is one of the major components of proteinuria, contributing to renal tubulointerstitial injury during CKD. Cubilin is a novel endocytosic receptor mediating albumin reabsorbtion by renal proximal tubular cell (RPTC). However, it is not clear whether cubilin play an important role in tubulointerstitial injury induced by albuminuria.
Methods: Ten nephrotic syndrome patients and eight hematuric patients were collected into this study. In vivo, albumin deposition, the expressions of cubilin, MCP-1 and RANTES in kidney tissue were examined by immunochemstry. To generate cubulin RNAi, the complementary oligonucleotides (containing target nucleotides 4701–4719 of cubilin) were annealed and cloned into pSUPEREGFP RNAi system according to the manufacture. The expression of cubilin mRNA by HK2 cell was determined by RT-PCR. Endocytosis of FITC labeled human serum albumin (FITC-HSA) by HK2 was examined by fluorescent microscope. Cubilin, MCP-1 and RANTES expression were detected by Western blot.
Results: Under the light microscope, only a small quantity of albumin was found within the RPTCs in the hematuric patients; but a large amount of albumin were seen within the RTECs and interstitium in the nephrotic syndrome patients. Cubilin, MCP-1 and RANTES were weakly expressed in the hematuric patients; but very strongly expressed in the nephrotic syndrome patients. In vitro, cubilin expression was obviously inhibited by pSUPEREGFP-CUB. The expressions of cubilin mRNA and protein in HK2 cells were down-regulated by pSUPEREGFP-CUB. HSA-FITC uptake was lower in HK2 cells transfected with pSUPEREGFP-CUB than those of harboring vector (pSUPEREGFP). Both MCP-1 and RANTES expression were significantly suppressed following pSUPEREGFP-CUB treatment.
Conclusion: Excessive albumin reabsorption may have close relationship with up-regulation of MCP-1 and RANTES by RTECs. Suppression of cubilin could play a potential role in prevention for albumin-induced renal tubulointerstitial injury.

W-PO30038

CELLULAR MARKERS OF IMMUNOINFLAMMATORY ACTIVITY (CIRCULATING MONOCYTE AND POLYMORPHONUCLEAR CELLS BURST ACTIVITY AND PHAGOCYTOSIS, AND MONOCYTE DR EXPRESSION) IN PATIENTS WITH SEVERE SEPSIS AT THE 'PEAKS' AND 'VALLEYS' OF C-REACTIVE PROTEIN (CRP) PLASMA LEVELS

JOSÉ H. ROCCO SUASSUNA[1], JOSÉ LUIZ DE CARVALHO[2], MARCELA SOUSA[3], GERALDO PEREIRA, MARJORIE GUIMARAES
Immunopathology Laboratory And Renal Unit. Faculdade De Ciências Médicas. Universidade Do Estado Do Rio De Janeiro[1], Ioc-Fiocruz[2]

Using C-reactive protein (CRP) as a surrogate of the systemic inflammatory response, we were able to detect a dynamic oscillatory behavior of systemic inflammation in sepsis, showing that patients progress longitudinally through 'peaks' and 'valleys' of inflammatory activity. The purpose of this study was to refine the above observations by assessing the relationship of these 'peaks' and 'valleys' with immunological competence.
We studied a group of severe sepsis patients and examined the functional features and the phenotype of monocytes and neutrophils in association with the first 'peak' and the first 'valley', as revealed by daily CRP levels. Patients were clustered in groups: 11 in the 'peak' phase and 12 in the 'valley' phase. Results were contrasted to those from 12 healthy controls.
Using a functional flow cytometry assay, we found that early in the course of the inflammatory peak, there are significant functional defects of both cell types, as expressed by phagocytosis and oxidative metabolism. Using a quantitative assay to detect the expression of labeled targets by flow cytometry, we found an inverse correlation between functional features and the expression of an 'inflammatory' CD64 phenotype on monocytes and neutrophils. We also detected a profound defect on monocyte surface HLA-DR expression on both phases (figure). While controls expressed a median of 33,010 molecules per cell, patients of the peak and valley groups expressed 4526 and 8351 molecules per cell, respectively.
These results show that peaks of inflammation are also associated with defective immunological features. Attenuation of inflammation leaves the patient in an immunodeficiency 'window' that may provide a necessary truce in pro-inflammatory activity that leads to clinical recovery, but may also predispose to recurrent infection.



W-PO30039

T-CELL SUBPOPULATIONS IN CHILDREN WITH MINIMAL CHANGE NEPHROTIC SYNDROME

QIAN SHEN[1], CHING-CHING SEAH[1], SUK-HIANG LAU[1], RU-BING LIU[1], LI-KIANG TAN[1], CHANG-LI WEI[1], HUI-KIM YAP[1]
Department of Pediatrics, National University of Singapore, Singapore[1]

T-lymphocytes have been implicated in the pathogenesis of minimal change nephrotic syndrome (MCNS). The aim of this study was to determine if certain T-cell subpopulations were associated with susceptibility to the disease, or implicated in relapse of the nephrotic syndrome. The study group consisted of 18 children with MCNS in relapse (mean age 10.2 ± 6.1 years), 18 age-matched children with MCNS in remission (mean age 10.9 ± 5.6 years), and 18 age-matched healthy children followed up for unilateral renal abnormalities (mean age 9.9 ± 6.6 years) as controls. Three-color flow cytometry was performed to determine the percentages of the various peripheral blood T-cell subpopulations, and the results were expressed as mean ± SD in the table below. The Mann-Whitney U test was used to determine the significance of the difference between the groups.

CD3+CD8+	TCRαβ+	TCRγδ+	CD45RA+	CD45RO+	CD25+
Relapse	81.4 ± 15.0*	4.6 ± 3.1	72.4 ± 15.8	31.1 ± 11.5*	0.9 ± 1.0
Remission	81.3 ± 9.3*	3.2 ± 3.3	65.4 ± 13.3	35.9 ± 11.2*	0.4 ± 0.8
Controls	60.7 ± 16.3	3.5 ± 2.9	72.5 ± 11.5	22.0 ± 9.7	1.0 ± 1.7
CD4+	**TCRαβ+**	**TCRγδ+**	**CD45RA+**	**CD45RO+**	**CD25+**
Relapse	90.9 ± 19.4	1.6 ± 1.5	55.6 ± 21.6	40.3 ± 19.4	15.2 ± 9.2*#
Remission	93.9 ± 12.8	1.9 ± 4.1	46.6 ± 18.4	43.1 ± 13.7	5.3 ± 3.4
Control	96.8 ± 4.3	0.8 ± 0.6	51.1 ± 17.6	35.3 ± 16.2	4.8 ± 3.1

* Comparison with controls, $p < 0.05$
[#] Comparison with remission, $p < 0.001$

Children with MCNS both in relapse and remission had higher percentage of TCRαβ+CD8+ cells as well as CD45RO+CD8+ cells than controls ($p < 0.05$). Patients with nephrotic relapse had a significant increase in the percentage of CD25+CD4+ cells compared to those in remission and controls ($p < 0.001$). In conclusion, in MCNS, the increase in TCRαβ+CD8+ subpopulation and memory CD45RO+CD8+ subpopulation are consistent with previously described oligoclonal expansion of CD8+ cells in this disease. There is also evidence of activated CD25+CD4+ cells during nephrotic relapses, consistent with our previous finding of upregulation of Th2 cytokine producing cells.

W-PO30040

ACTIVATION OF INNATE IMMUNITY IN A RAT MODEL OF CHRONIC CYCLOSPORINE NEPHROPATHY

CAN LI[1], SUN WOO LIM[2], KYUNG OK AHN[2], JIN KIM[3], IN SUNG MOON[4], CURIE AHN[5], CHUL WOO YANG[2]
Internal Medicine, YanBian University Medical College[1], Internal Medicine, The Catholic University of Korea, Korea[2], Anatomy, The Catholic University of Korea, Korea[3], Surgery, The Catholic University of Korea, Korea[4], Internal Medicine, Seoul National University College of Medicine, Korea[5]

Background: There is growing evidence that innate immunity plays a potential role in the pathophysiology of injury-associated kidney diseases. This study was performed to evaluate the influence of cyclosporin A (CsA)-induced renal injury on innate immunity in an experimental model of chronic CsA nephropathy.
Methods: Activation of innate immunity was evaluated in terms of toll-like receptor (TLR) 2 and TLR4 mRNA and protein expression, TLR signal pathway, costimulatory molecules, and maturation of dendritic cells. Daily CsA treatment (15 mg/kg per day) for 28 days in Sprague-Dawley rats induced characteristic lesions that were remarkably similar to chronic CsA nephropathy in humans.
Results: Long-term CsA treatment upregulated TLR2 and TLR4 mRNA and protein expression, accompanied by increased MYD88, NF-kB and AP-1 expression. CsA-treatment also increased the expression of MHC class II antigen and costimulatory molecules (B7-1, B7-2). Double labeling of markers of dendritic cells and MHC class II antigen revealed that matured dendritic cells are increased in CsA-treated rat kidneys.
Conclusion: These findings indicate that chronic CsA nephropathy is closely associated with increased innate immunity.

W-PO30041

ACTIVATION OF INNATE IMMUNITY IN ISCHEMIA-REPERFUSION RAT KIDNEY

CAN LI[1], SUN WOO LIM[2], JIN KIM[3], BYUNG KEE BANG[2], CHUL WOO YANG[2]
Internal Medicine, YanBian University Medical College[1], Internal Medicine, The Catholic University of Korea, Korea[2], Anatomy, The Catholic University of Korea, Korea[3]

Background: Immune system plays a key role in the pathophysiology of ischemia-reperfusion (I/R) injury, but the influence of I/R injury on innate immunity in the kidney has yet to be illustrated.

Methods: I/R injury was induced in Sprague Dawley rats by clamping both renal arteries for 45 min, and the rats were sacrificed one, three, five, and seven days later. Influence of I/R on innate immunity was evaluated in terms of the expression of toll-like receptor (TLR) 2 or TLR 4 mRNA, the level of the TLR ligand (heat shock protein [HSP] 70), and maturation of dendritic cells (double label immunohistochemistry of dendritic cells for MHC class II antigen).
Results: I/R injury increased TLRs (TLR 2 and TLR 4) mRNA and protein expression and the production of endogenous TLR ligand (HSP 70), and they were mainly observed on the renal tubular cells. I/R injury increased not only the numbers of dendritic cells (OX 6-positive cells) but also the production of MHC class II antigen in dendritic cells (OX 62-positive cells, 27 ± 2 vs. 13 ± 1, $P < 0.01$), suggesting maturation of these cells. Activation of innate immunity was observed at day one, peaked at day three to five after I/R injury, and thereafter gradually decreased.
Conclusion: I/R injury rapidly activates the innate immune response.

W-PO30042

ANTI-DNA ANTIBODIES FROM PATIENTS WITH LUPUS NEPHRITIS INDUCE IL-6 EXPRESSION IN RENAL TUBULAR EPITHELIAL CELLS

SUSAN YUNG[1], RYAN CW TSANG[1], JACK KH LEUNG[1], TAK MAO CHAN[1]
Department of Medicine, University of Hong Kong, Hong Kong SAR[1]

There is limited data on the pathogenesis of tubulo-interstitial lesions in lupus nephritis. In this study we investigated the effect of human anti-DNA antibodies on IL-6 synthesis and cellular functions in proximal renal tubular epithelial cells (PTEC).
PTEC were isolated from renal cortical tissue by differential sieving and digestion with collagenase. Paired polyclonal anti-DNA antibodies were isolated from 15 lupus nephritis patients during active disease and remission using DNA-cellulose affinity chromatography. Cell morphology was assessed by phase contrast microscopy, and cell proliferation and viability by MTT assay and lactate dehydrogenase release respectively. IL-6, IL-1beta, and TNF-alpha were determined in culture supernatants using commercial ELISAs.
Incubation of PTEC with anti-DNA antibodies (10 micrograms IgG/ml) for time periods up to 24 h induced IL-6 secretion in a time-dependent manner (0.5 +/– 0.1, 8.0 +/– 3.1, and 12.4 +/– 4.7 ng/microgram cellular protein for control IgG, anti-DNA antibodies during inactive disease, and anti-DNA antibodies during active disease respectively, p 0.01 compared to control), which was dependent on de novo mRNA and protein synthesis. Induction of IL-6 secretion was accompanied by alterations in cell morphology, proliferation (p 0.05), and viability (p 0.05), as well as increased secretion of IL-1beta (p 0.05) and TNF-alpha (p 0.05). IL-6 induction by anti-DNA correlated with circulating anti-DNA antibody levels and their binding to cultured PTEC (Spearman $r = 0.561$ and 0.576 respectively, p 0.001 for both). Data from inhibition experiments using neutralizing antibodies to IL-6 and TNF-alpha, and IL-1 receptor antagonist showed that during active disease IL-6, IL-1beta, and TNF-alpha interacted synergistically with anti-DNA antibodies to induce PTEC IL-6 secretion, but TNF-alpha was not involved in such induction during remission.
Our data demonstrate that anti-DNA antibodies from patients with lupus nephritis can induce IL-6 production in PTEC, through distinct mechanisms that vary according to disease activity.

W-PO30043

SWITCH FROM PROTEASOME TO IMMUNOPROTEASOME IN CIRCULATING LYMPHOMONOCYTES OF PATIENTS WITH IGA NEPHROPATHY

ALESSANDRO AMORE[1], ALDA ALFARANO[2], MONICA CHIESA[1], ALESSANDRA MOLLO[1], STEFANIA TURCATO[1], LAURA GHISELLINI[1], PAOLA CIRINA[1], LUCA ROASIO[1], GIOVANNI CONTI[1], LICIA PERUZZI[1], PIERANGELO TOVO[2], ROSANNA COPPO[1]
Nephrology Dialysis Transplantation, Regina Margherita University Hospital, Torino Italy[1], Ped Clin University Hospital, Torino Italy[2]

Proteasomes play a key role in the degradation of proteins, hence regulating activation of transcription factors, production of pro-inflammatory cytokines and intracellular processing of antigens with generation of MHC class I restricted peptides. Proteasomes are detected in the majority of the cells. A few mature immunocompetent cells, such as mature dendritic cells, upon the effect of interferons and undergo a switch from proteasome (PS) to immunoproteasome (iPS), by replacing three cathalitic subunits provided with chymotrypsin-, trypsin-like and peptidyl-glutamyl-peptide hydrolysing activity with three peptides called low-molecular weight (LMP) 2 and 7 and endopeptidase complex-like (MECL-1), then acquiring catalytic properties optimal for MHC-I peptide presentation.
We aimed to investigate whether in IgA nephropathy (IgAN), a disease with complex immune system abnormalities, proteasomes of peripheral blood mononuclear cells (PBMC) present with signs of switch to iPS.
PBMC from 20 patients with IgAN and 20 healthy controls, isolated by gradient centrifugation, were tested by quantitative Taqman analysis for LMP2, LMP7 and MECL-1 mRNA expressions. In parallel, levels of circulating aberrantly glycosylated IgA1 with increased GalNAc exposure, which is presently considered the major immunological abnormality of these patients, were tested (binding of IgA1 isolated by Jacalin affinity chromatography to Helix Aspersa and to Vicia Villosa).
Table reports the normalized data of mRNAs. p values refer to the comparison between healthy controls and IgAN data by Student s t-test.

	LPM-2	LPM-7	MECL-1
Healthy controls	0.98 ± 0.17	1.16 ± 0.40	1.40 ± 0.24
IgAN patients	1.53 ± 0.26*	1.83 ± 1.46*	1.61 ± 0.51*

* $p < 0.0001$ vs healthy controls

Patients showed also significantly high levels of IgA1 exposing GalNAc.
Our results indicate for the first time that peripheral immunocompetent cells from IgAN patients present with a switch from proteasome to immunoproteasome, with possible profound implications in antigen presentation. This finding opens the question whether the switch to immuneproteasome in circulating PBMC of IgAN patients can be considered a sign of activation of innate immunity.

W-PO30044

TISSUE LEVEL EVIDENCE FOR HIGHER INTENSITY OF INFLAMMATION IN THE ATHEROSCLEROTIC PLAQUES OF UREMIC PATIENTS

MARIE-LUISE PAMELA GROSS[1], HANS-PETER MEYER[2], HEIKE ZIEBART[3], IRINA BERGER[3], KATERINA NEUMANN[3], KERSTIN AMANN[4], EBERHARD RITZ[5]
Institute of Pathology[1], Institute of Mineralogy, University of Heidelberg[2], Institute of Pathology, University of Heidelberg[3], Institute of Pathology, University of Erlangen[4], Dept. of Internal Medicine, Nephrologic Division, University of Heidelberg, University of Erlangen[5]

Coronary calcification, as assessed in vivo, has been shown to be a potent predictor of cardiac events. In renal patients both prevalence and intensity coronary and aortic calcification are markedly increased. There is an ongoing discussion whether the pattern of calcification is different in renal and non-renal patients and whether coronary calcification, as assessed in vivo, reflects intimal calcification, medial calcification (or both).
The coronary and aortic samples, obtained at autopsy at standardised sites, of 11 uremic patients and 8 non-uremic patients were analysed in the present study. Samples of coronary arteries as well as aortic samples were examined by electron and light microscopy and by immunohistochemistry using antibodies against CD 68, osteocalcin, CRP, C5b-9, TGF-β, ET-1, collagen IV, MMP2, VEGF. Scores were obtained by 2 investigators blinded with respect to the diagnosis (Score 0–4). Probes of directly adjacent tissue and chemical analyses were carried out with a Leo 440 Scanning electron microscope.
The media of coronaries of uremic patients (media: $16.6 \pm 10.6\%$ of area) was significantly more calcified than that of non-uremic controls (media: $3.8 \pm 2.31\%$). Calcification of the intima was not different between the groups. In the aorta calcification was significantly more pronounced in the intima and media of uremic patients (intima: 22.5 ± 7.9 %, media: $11.0 \pm 7.9\%$) compared to non-uremic controls (intima: $10.4 \pm 5.2\%$, media: $4.4 \pm 2.2\%$). On the protein level expression of osteocalcin, CRP, TGF-β and collagen IV was significantly increased in the media of aorta and coronaries of uremic patients compared to non-uremic patients with aquivalent calcifications in both sites. C5b-9 was significantly increased only in the media of the coronary, but not in the aorta compared to non-uremic controls.
Vascular lesions of uremic patients are associated with significantly more marked expression of indicators and/or mediators of inflammation.

W-PO30045

GLOMERULAR IMPAIRMENT BY HEMOPEXIN IS INHIBITED BY EXTRACELLULAR ATP

WINSTON W. BAKKER[1], MARIJKE M. FAAS[1], RIANNE M. JONGMAN[1], THEO BORGHUIS[1]
Dept of Pathology, University Hospital Groningen, The Netherlands[1]

Circulating factors are believed to play a role in the pathogenesis of minimalchange disease (MCD).
One of such factors may be an active isoform of plasma hemopexin (Hx), which shows a relative high protease activity in subjects with MCD in relapse versus remission. In vitro and in vivo Hx affects glomerular extra cellular matrix molecules (ECM's). Extracellular ATP may act as the "natural" inhibitor of Hx. To test Hx inhibition by extracellular ATP we now compared plasma from subjects with MCD in relapse (n = 10) with or without supplementation of ATP (2 mM in PBS), with that from subjects with preeclampsia (PE, n = 9)or from healthy control donors (n = 8) for their capacity to affect glomerular ECM's.(mean plasma ATP levels of PE subjects are significantly higher as compared with plasma from healthy donors) Kidney sections, immunostained for glomerular ECM's after incubation with diluted plasma from patients with either MCD, PE or healthy controls, were scored for reaction product using standard.
Methods: It appeared that exclusively MCD relapse plasma was able to significantly affect glomerular ECM's. When inactive plasma samples from PE subjects or control donors were treated with alkaline phosphatase (AP, 25.0 U/ml)) or apyrase (1.0 U/ml) and tested again for their protease activity, significant loss of ECM occurred. It is concluded that dephosphorylation of inactive plasma Hx by these enzymes stimulated the conversion into the active isoform of Hx in the plasma samples tested. Incubation of AP or apyrase alone with kidney tissue did not affect glomerular stainability for ECM.
The mechanism of phosphorylation of Hx by extracellular ATP and its putative role in vivo remains to be established.

W-PO30046

ROSIGLITAZONE, AN AGONIST OF PEROXISOME-PROLIFERATOR ACTIVATED RECEPTOR-GAMMA, AMELIORATES CISPLATIN NEPHROTOXICITY THROUGH THE INHIBITION OF TNF-α/NF-κB ACTIVATION

SUNG-KWANG PARK[1], SIK LEE[1], WON KIM[1], SANG-OK MOON[1], MI JEONG SUNG[1], DUK HOON KIM[1], KYUNG PYO KANG[1], SUNG KYEW KANG[1]
Department of Internal Medicine, Renal Regeneration Laboratory, Research Institute of Clinical Medicine, Chonbuk National University Medical School[1]

Background: Recently, it was suggested that inflammatory mechanisms play an important role in the pathogenesis of cisplatin nephrotoxicity. Agonists of the peroxisome proliferator-activated receptor gamma, such as rosiglitazone, were recently demonstrated to regulate inflammation by modulating the production of inflammatory mediators and adhesion molecules. The purpose of this study was to determine the protective effects of rosiglitazone on cisplatin nephrotoxicity and to explore the mechanism of its reno-protection.
Methods: The effects of treatment with cisplatin alone and in pretreatment with rosiglitazone in mice on renal function, histologic findings, aquaporin 2 (AQP2) expression, and tumor necrosis factor (TNF)-α level were investigated. The effect of rosiglitazone on nuclear factor (NF)-κB activity was examined in vitro using cultured HK-2 cells.
Results: Rosiglitazone significantly decreased both the renal functional and histologic damage after cisplatin injection. Pretreatment with rosiglitazone reduced the increased renal and systemic levels of TNF-α and down-regulated the adhesion molecules expression in addition to the infiltration of inflammatory cells after cisplatin injection. Rosiglitazone also recovered the decreased AQP2 expression after cisplatin treatment. In addition, pretreatment with rosiglitazone inhibited the activation of the p65 subunit of nuclear factor (NF)-kB in cultured HK-2 cells.
Conclusions: These results showed that pretreatment with rosiglitazone attenuated cisplatin-induced renal damage through the inhibition of TNF-α overproduction and NF-κB activation.

W-PO30047

INTERACTION OF CIRCULATING FACTORS ASSOCIATED WITH CORTICOSTEROID RESPONSIVE NEPHROTIC SYNDROME EXPRESSION OF HEMOPEXIN IN BLOOD CELLS AFTER STIMULATION WITH INTERLEUKIN-10 IN VITRO IS INHIBITED BY PREDNISOLONE

WINSTON W. BAKKER[1], RIANNE JONGMAN[1], THEO BORGHUIS[1]
Dept. Of Pathology, University Hospital Groningen, The Netherlands[1]

An active isoform of plasma hemopexin (Hx) as well as interleukin-10 (IL-10) may be implicated in the pathogenesis of corticosteroid responsive nephrotic syndrome (CRNS). Thus, subjects with CRNS in relapse versus remission showed enhanced protease activity in their plasma Hx. An enhanced mRNA signal for IL-10 in peripheral blood mononuclear cells (PBMC) has also been observed in these patients. To investigate a possible relationship between IL-10, Hx expression of PBMC and potential corticosteroid sensitivity of this putative interaction, we stimulated PBMC from healthy donors (n = 11; 1×10^6 cells/ml) in vitro with IL-10 (10 ng/ml) with or without prednisolone (1.0 μM) for 18 hrs under serum free conditions. After discontinuation of the cultures cytospins were immunostained using monoclonal anti HxIgG and peroxidase conjugated anti mouse antibodies according to standard
Methods: Numbers of positive staining cells were counted. A part of the cell cultures was prepared for flow cytometry according to standard procedures. The results of the cytospins show increased staining of IL-10 stimulated cells as compared with non stimulated cells ($80 \pm 5\%$ versus $45 \pm 9\%$ positive cells ($p \leq 0.01$). Supplementation of prednisolone 1 μM to the IL-10 stimulated cultures showed a significant decrease of staining ($30 \pm 6\%$). Also the flow cytometry data show significant relative increase of Hx expression after IL-10 stimulation as compared to unstimulated cells, whereas this relative increase was significantly reduced after supplementation with 1 μg/ml prednisolone. (267% and 128% respectively; $P < 0.01$) Addition of 10 μM prednisolone to IL-10 stimulated cells resulted in further loss of Hx expression. It is concluded that stimulation of normal human PMBC by IL-10 results in enhanced expression of Hx by these cells, which can be significantly suppressed by prednisolone in vitro. The potential relevance of this interaction in CRNS in vivo requires further investigation.

THEME 4: DIALYSIS

Cardiovascular Risk Factors

W-PO40001

C-REACTIVE PROTEIN FOR PREDICTING ACUTE CORONARY SYNDROME IN HEMODIALYSIS PATIENTS

HASE-H[1], JOKI N[1], INISHI Y[1], ISHIKAWA H[1], TANAKA Y[1], TAKAHASHI Y[1], IMAMURA Y[2], NAKAMURA M[3], MOROI M[3]
Division of Nephrology Toho University Ohashi Hospital[1]*, Hemodialysis Center Nissan Tamagawa Hospital*[2]*, Division of Cardiology Toho University Ohashi Hospital*[3]

Background: Acute coronary syndrome (ACS) is high prevalence in hemodialysis patients. We hypothesized that C-reactive protein might be an independent predictor of a future ACS in hemodialysis patients.
Methods: We prospectively evaluated ability of myocardial perfusion imaging performed under pharmacologic stress to predict 2-year outcomes in 86 hemodialysis patients, specifically thallium-201 single-photon emission computed tomography using high-dose adenosine triphosphate as the stressor, and the effect of myocardial perfusion SPECT and levels of C-reactive protein (CRP) for long-term risk of ACS.
Results: Twenty-three (27%) patients had ACS during follow-up. Time until occurrence of ACS showed a significant, independent association with an elevated baseline CRP ($\beta = -5.714$, $p < 0.01$), known coronary artery disease ($\beta = -4.294$, $p < 0.01$), and a reversible myocardial perfusion defect ($\beta = -5.906$, $p < 0.01$). The positive and the negative prediction values of CRP at 3 months before ACS (cut-off point: 0.55 mg/dL) by an analysis based on the 'best cut-off' in identified on the receiving operation characteristic (ROC) curves were higher than those of baseline CRP (cut-off point: 0.35 mg/dL) in all patients (82.6% vs.

60.9%, 96.8% vs. 84.1%, respectively) and in patients with normal myocardial perfusion (100% vs. 60.6%, 98.0% vs. 82.4%, respectively).

Conclusions: Circulatory elevated CRP was significantly and closely associated with not only the development of coronary atherosclerosis and vulnerability of coronary plaque but also occurrence of ACS in hemodialysis patients. These results identify an important role for CRP in the pathophysiology of coronary plaque development/progression in hemodialysis patients.

W-PO40002

HYPERLIPIDEMIA IN CHILDREN ON CHRONIC HEMODIALYSIS: EFFECT OF ORAL L-CARNITINE ON LIPID PROFILE

REZA KHAZAEI MAHMOOD[1], ALI DERAKHSHAN ALAMDARLOO[2]
Mashad Faculty of Medicine – Islamic Azad University, Mashad, Khorasan, IRAN[1], Pediatric Nephrology, Nemazi Hospital, Shiraz, Fasrs, Iran[2]

Hyperlipidemia (HL), especially when started during early childhood will increase the risk of atherosclerosis. It is also a major risk factor for allograft nephropathy and post-transplant HL, so its treatment is highly suggested. In this study we evaluated the effect of L-carnitine on the lipid profile of children with end stage renal disease (ESRD) on maintenance hemodialysis (HD) after a 3-month period. Twelve out of 15 children; i.e. 80% had abnormal lipid profile at the beginning of the study. HDL-C level increased in all of them following the treatment (P < 0.001). No significant change occurred in the level of triglyceride, cholesterol, LDL-C, VLDL-C, but the ratio of TG : HDL-C as a major risk factor for cardiovascular disease decreased significantly (P = 0.004). In conclusion dyslipidemia is common in hemodialysed children. In our study, short term L-carnitine administration (3 months) had major effect on HDL-C level increment; a longer administration may affect other components, too.

Lipid profiles In children on chronic hemodialysis: before and after 3 months oral L-carnitine therapy.

	pre-treatment	post-treatment	P. value
Total Cholestrol(mg/dl)	150.8 (±34.1)	157.5 (±25.3)	>0.05
Triglyceride (mg/dl)	194.2 (±53.8)	182.2 (±48.7)	>0.05
HDL-C (mg/dl)	22.8 (±5.5)	40.8 (±6.2)	<0.001
VLDL-C (mg/dl)	38.8 (±8.4)	36.3(±9.1)	>0.05
LDL-C (mg/dl)	89.2 (±25.9)	80.3(±24.3)	>0.05
TG/HDLC	8.97 (±3.4)	4.9 (±1.7)	0.004

•• : mean (±SD)

W-PO40003

CLOSE RELATIONSHIP OF CAROTID INTIMA-MEDIA THICKNESS WITH LEFT VENTRICULAR HYPERTROPHY AND EJECTION FRACTION IN END-STAGE RENAL DISEASE PATIENTS UNDERGOING HEMODIALYSIS TREATMENT

HAMID NASRI, MD[1], AMIR SAID ALIZADEH NADERI, MD[2]
Shahrekord University of Medical Sciences[1], University of Hamburg[2]

Background: Two principle findings of cardiovascular disease in end-stage renal disease patients undergoing regular hemodialysis is left ventricular hypertrophy (LVH) and arterial disease due to rapidly progressive atherosclerotic vascular disease that can be characterized by an enlargement and hypertrophy of arteries (intima-media complex thickening). In this study we sought to study the relationship of left ventricular hypertrophy with intima-media complex thickening (IMT) in end-stage renal disease patients undergoing regular hemodialysis.

Patients and Methods: This cross-sectional study was done on sixty-one patients with end-stage renal disease (ESRD) undergoing regular hemodialysis treatment (F = 23, M = 38) consisting of 50 non diabetic hemodialysis patients (F = 20, M = 30) and 11 diabetic hemodialysis patients (F = 3, M = 8). For all patients echocardiography was done and carotid-intima-media thickness was measured by B-mode ultrasonography.

Results: There was positive correlation between stages of LVH with duration of hemodialysis treatment. Positive correlation between stages of LVH with stages of hypertension was seen. Moreover, significant correlation between stages of LVH with carotid-IMT and also positive correlation between stages of LVH with presence of chest pain was found. More thickening of intima-media complex in diabetic group, and association of diabetes mellitus with the presence of chest pain as well as positive correlation between stages of HTN with IMT was also demonstrated. Moreover, linear inverse correlation between IMT with percent of LV ejection fraction was observed.

Conclusion: Thickening of intima-media complex is more evident in hemodialysis subjects with LVH, when there is an LVH; the IMT is similar in severity to the LVH.

W-PO40004

INCORRECT DRY WEIGHT – A POTENTIAL RISK FACTOR OF UREMIC CARDIOMYOPATHY

C KUHN[1], A KUHN[1], K RYKOW[1], R STEUDTE[1], B OSTEN[1]
Department of Internal Medicine II, University of Halle-Wittenberg[1]

Impaired myocardial function is an often seen clinical picture in chronic hemodialysis patients. There are many factors in hemodialysis patients leading to cardiac dysfunction. However, the contribution of each factor is still not clear. We investigated the dry weight of 35 chronic hemodialysis patients by means of determination of extravascular lung water index (ELWI) as a volume parameter to identify fluid overload. Therefore patients were connected to the PiCCO-system (Fa. Pulsion) before starting hemodialysis treatment. The following hormones were measured before and after hemodialysis: brain natriuretic peptide (BNP), aldosterone, renin.

Patients characteristics and hemodynamic parameters are shown in the table. Elevated BNP pre-dialytic serum levels in 12 of 14 patients were found but there was no significant change post-dialysis. In 22 of 35 patients (63%) strong elevated values of ELWI pre-dialytic were measured indicating volume overload although the patients were near their clinical determined dry weight. With the aid of ELWI the clinical estimated dry weight was reduced and a significant increase of the left ventricular contractility index was observed indicating improved ventricular performance.

Our data support the importance of a correct dry weight because volume overload leads to significant impaired cardiac performance as shown and plays a major role in the genesis of uremic cardiomyopathy.

Table 1

parameter	pre-dialysis	post-dialysis
age (y)	56 ± 13.1	
Δ dry weight – weight pre-HD	278.8 ± 876.7	
LV-enddiastolic volume (ml)	137.2 ± 51.6	
BNP (pg/ml)	746.9 ± 980.2	903.2 ± 1299.0
aldosterone (pmol/l)	213.7 ± 104.2	155.5 ± 65.2
renin (ng/ml)	0.93 ± 1.2	4.5 ± 10.0
cardiac index (ml/min/m^2)	3.7 ± 0.9	3.47 ± 0.7
global ejection fraction (%)	20.4 ± 5.2	19.8 ± 4.8
stroke volume index (ml/m^2)	51.2 ± 14.4	44.3 ± 10.8*
mean arterial pressure (mmHg)	104.8 ± 16.5	87.4 ± 19.6*
systemic vascular resistance index (dyn*s*cm^{-5}*m^2)	2244.9 ± 680.9	1907.1 ± 614.4*
heart rate (beats/min)	72.3 ± 12.7	79.6 ± 15.1*
left ventricular contractility index (mmHg/s)	1055.2 ± 287.6	1323.6 ± 343.1*
ELWI (ml/kg)	11.1 ± 6.2	10.7 ± 6.0

p < 0.05

W-PO40005

ISOLATED SYSTOLIC HYPERTENSION AND INCREASED PULSE PRESSURE IN PATIENTS WITH CHRONIC HEMODIALYSIS

MANUEL TORRES-ZAMORA[1], MARÌA ANGÈLICA JAIME SOLIS[1], SIGFRIDO HUERTA[2], LUIS MAURO ARTEAGA[1], SILVESTRE GAITÁN[1], LEOPOLDO VÁSZQUEZ[1], OSCAR MANZANO[1], HÈCTOR ROMERO[1], EDWIN MARKA[1], JAVIER VIVALDO[3]
Hospital Dalinde[1], Isste[2], Universidad Autònoma Metropolitana[3]

Isolated systolic hypertension and increased pulse pressure are associated with cardiovascular complications and death in patients with chronic hemodialysis. This study was conducted in order to examine the prevalence of isolated systolic hypertension and increased pulse pressure in patients with chronic hemodialysis.

Methods: Systolic and diastolic pressures were recorded before and after three weekly sessions of hemodialysis in 148 adult patients undergoing maintenance hemodialysis. Pulse pressure was quantified as the difference between systolic and diastolic pressure. Diastolic hypertension was defined as blood pressure >140/

90 mmHg., whereas systolic hypertension was defined as systolic pressure >140 mmHg and diastolic of 90 mmHg or less. The volume of fluid removal during dialysis in volumetric hemodialysis machines was calculated to reach the dry weight.

Results:

	n (%)		Hypertensive patients	
Total patients	148 (100)		Predialysis	Postdialysis
Systolic hypertension	84 (56.7)	Systolic (mmHg)	149.6 ± 20	140 ± 20*
Diastolic hypertension	11 (7.4)	Diastolic (mmHg)	79.7 ± 12	78.8 ± 11
Normotensive	53 (35.8)	Pulse (mmHg)	69.8 ± 19	61.7 ± 18*

* $p < 0.01$ vs predialysis Ultrafiltration (ml) 3007 ± 1076

The results showed that systolic hypertension was the most frequent type of hypertension, 84 patients (56.7%); only 11 patients (7.4%) had diastolic hypertension and 53 patients (35.8%) were normotensive. The hypertensive population had a mean systolic blood pressure of 149.6 ± 20 mmHg before dialysis which decreased to 140 ± 20 mmHg ($p < 0.01$) after it. Predialysis pulse pressure was 69.8 mmHg and the postdialysis pulse pressure decreased to 61.7 ± 18 mmHg ($p < 0.01$).

Conclusions: The high prevalence of systolic hypertension and an increase of pulse pressure may be the cause of cardiovascular morbidity and death in chronic hemodialysis.

W-PO40006

STUDY OF ACUTE PHASE PROTEINS IN RELATION TO CARDIOVASCULAR DISEASE IN PATIENTS ON CHRONIC HEMODIALYSIS

DIMITRIOS KIRMIZIS[1], APOSTOLOS TSIANDOULAS[1], EVANGELIA KOUTOUPA[2], MARIA PANGALOU[3], PARASKEVI ROZI[2], MARIA PROTOPAPPA[3], KONSTANTINOS BARBOUTIS[1]

Department Of Nephrology, Serres General Hospital[1], Laboratory Of Microbiology, Serres General Hospital[2], Laboratory Of Hematology, Serres General Hospital[3]

Objective: To investigate the differences in acute phase protein levels among patients with end-stage renal disease (ESRD) on chronic hemodialysis treatment (HD) with and without cardiovascular disease (CVD).

Methods: We studied 36 patients with ESRD (19 males, mean age 66 ± 9 years), stabilized on HD (mean duration 58 months, range 6–210). 10 patients (28%) had a history of CVD (6 males, mean age 72 ± 4 years). Serum high-sensitivity CRP (hsCRP) and lipoprotein (a) (Lpa), as well as plasma fibrinogen (PF) and routine biochemistry were determined.

Results: hsCRP, Lpa and PF were significantly higher in patients with CVD compared to patients without CVD (0.79 ± 0.5 vs 0.75 ± 0.4 mg/dL, $p = 0.004$, 0.45 ± 0.27 vs 0.37 ± 0.27 mg/dL, $p < 0.001$ and 318.7 ± 63.3 vs 296.4 ± 86.5 mg/dL, $p < 0.0001$, respectively). Patients with CVD had lower serum protein and albumin levels compared to other patients (6.9 ± 0.6 vs 7.2 ± 0.5 g/dL, $p < 0.001$, and 3.9 ± 0.4 vs 4.1 ± 0.2 g/dL, $p < 0.001$, respectively). hsCRP exhibited a significant positive correlation with PF ($R = 0.47$, $P < 0.001$), Lpa ($R = 0.19$, $P < 0.001$) and age ($R = 0.48$, $P < 0.001$) and a significant negative correlation with serum albumin ($R = 0.44$, $P < 0.0001$) and HD duration ($R = 0.18$, $P < 0.001$). PF appeared a significant correlation to the age and CVD ($R = 0.18$, $P < 0.0001$ and $R = 0.14$, $P < 0.0001$ respectively). Multiple regression analysis for patients with CVD showed that the strongest independent predictors of hsCRP are PF, serum albumin, HD duration, age, and Lpa in this order. Multiple regression analysis for patients with CVD showed that the strongest independent predictors of PF are hsCRP, HD duration, age, Lpa and serum albumin, in this order.

Conclusions: Markers of inflammation and coagulation appear to correlate with one another and with CVD in patients on HD. Although the exact pathogenetic sequence is not precisely known, predominant among the correlations in our patients was the association between hsCRP and PF, pointing towards a close relation of inflammatory mechanisms with coagulation.

W-PO40007

PLASMA D-DIMERS ARE A STRONG INDEPENDENT PREDICTOR OF CARDIOVASCULAR DISEASE IN PATIENTS ON CHRONIC HEMODIALYSIS

DIMITRIOS KIRMIZIS[1], APOSTOLOS TSIANDOULAS[1], MARIA PANGALOU[2], MARIA PROTOPAPPA[2], KONSTANTINOS BARBOUTIS[1]

Department of Nephrology, Serres General Hospital[1], Laboratory Of Hematology, Serres General Hospital[2]

Objective: To investigate whether plasma D-dimers, as marker of the turnover of fibrin and the activation of the haemostatic system, relates to cardiovascular disease (CVD) in patients with end-stage renal disease (ESRD) on chronic hemodialysis treatment (HD), in comparison to other established predictors of CVD.

Methods: We studied 71 patients with ESRD (43 males, mean age 65 ± 11 years), stabilized on HD (mean duration 54 months, range 6–210). 14 patients (20%) had diabetes mellitus (DM) and 22 patients (31%) had a history of documented cardiovascular CVD (16 males, mean age 70 ± 6 years). D-dimers (PD) and fibrinogen (PF) in citrated plasma was determined, along with the routine biochemistry. The results were assessed in relation to CVD, arterial hypertension (AH), age and duration of HD (months).

Results: PD and PF were significantly higher in patients with CVD compared to patients without CVD (337.9 ± 81.7 vs 317.8 ± 80.0 mg/dL, $p < 0.0001$ and 595.7 ± 387.0 vs 313.9 ± 108.1 ng/mL, $p < 0.0001$, respectively). PD exhibited a significant positive correlation with the age of the patients and CVD ($R = 0.431$, $P < 0.0001$, and $R = 0.489$, $P < 0.0001$, respectively) and a significant negative correlation with the HD duration ($R = 0.296$, $P < 0.0001$). PF appeared a significant, but more weak compared to PD, correlation to the age and CVD ($R = 0.04$, $P < 0.0001$ and $R = 0.07$, $P < 0.0001$ respectively). PF also exhibited a significant, although weaker compared to PD, negative correlation with the HD duration ($R = 0.04$, $P < 0.0001$). Multiple regression analysis for patients with CVD showed that the strongest independent predictors of CVD are PD, AH, age, sex, serum protein concentrations, duration of HD, PF and serum albumin concentrations in this order.

Conclusions: PD are found to be significantly higher in patients with CVD on HD. PD exhibited a parallel pattern with PF but a more significant correlation with CVD compared to PF. This might be of prognostic significance in this patient population.

W-PO40008

ASSOCIATION BETWEEN INFLAMMATORY INDICES AND CAROTID ATHEROSCLEROTIC DISEASE IN PATIENTS UNDERGOING HEMODIALYSIS

APHRODITE AVDELIDOU[1], ANGELIKI THEODORIDOU[1], EVAGGELOS DESTANIS[1], IPPOLITI IKONOMOU[1], ATHANASIOS DIMITRIADIS[1], DIMITRIOS GREKAS[1]

Ahepa University Hospital Thessaloniki Greece[1]

Inflammation is considered a major risk factor for cardiovascular morbidity and mortality. It is well known that inflammation mechanisms are being activated during hemodialysis treatment. Aim of our study is to investigate the association of carotid atherosclerotic disease to hemodialysis patients with reference to serum CRP and albumin levels.

Patients-Methods: 37 patients (19 males and 18 females) undergoing chronic hemodialysis and 30 healthy individuals (sex and age matched) were included in our study. All hemodialysis patients and healthy controls underwent echo-doppler carotid artery examination. Our study included determination of the number and the dimensions of all the atherosclerotic plaques and measurement of carotid lumen diameter. Blood samples had been taken for the determination of serum CRP and albumin levels in both studied groups.

Results: Both lumen diameter and dimensions of the atherosclerotic plaques were significantly increased in hemodialysis patients compared to healthy controls ($p < 0.01$ and p 0.001 respectively). Positive correlation was found between serum CRP levels and dimensions of the atherosclerotic plaques, as well as between CRP levels and carotid lumen diameter ($p < 0.001$) in the patients' group. Serum albumin levels were inversely correlated with the dimension of the atherosclerotic plaques ($p < 0.001$). There was also a significant positive correlation between carotid lumen diameter and dimensions of atherosclerotic plaques in chronic hemodialysis patients ($p < 0.001$).

Conclusions: 1. Atherosclerotic lesions are more prevalent in chronic hemodialysis patients compared with healthy controls. 2. Inflammatory indices such as high CRP levels and low serum albumin levels are strongly associated with carotid atherosclerosis in hemodialysis patients.

W-PO40009

ASSOCIATION BETWEEN NUTRITION AND VASCULAR RISK FACTORS IN HEMODIALYSIS PATIENTS WITH DIFFERENT TYPES OF MEMBRANES

<u>LYGERI PAULOS SOUBASSI</u>[1], EMMANUEL PAPADAKIS[1], STELIOS NIKOLAOS ZEREFOS[1], ILIAS THEODOROPOULOS[1], GEORGE POULOS[1], ALEKSANDROS KOUVELIS[1], SIMEON KAPNISIS[1], IOANNIS TSAPAKIDIS[1], GRIGORIOS DAGLAS[1], DIMITRIOS VALIS[1], NIKOLAOS STYLIANOS ZEREFOS[1]

Renal Unit, Hygeia Hospital, Greece[1]

Atherosclerotic cardiovascular disease and malnutrition are widely recognized as leading causes of the increased morbidity and mortality observed in uremic patients.

Aim: The aim of this study was to evaluate the influence of nutrition on the s.levels of several vascular risk factors as: Lp (a), fibrinogen (FNG), iPTH, IL-6, IL-8, total leptin in hemodialysis (HD) patients (pts).

Methods: They were dialyzed with two different types of membranes, cellulose based (CBM) and synthetic polysulfone (SPM). The study included 40 pts (26 M and 14 F) aged 52.4 + −11, who have been on regular bicarbonate HD for 96 + −65 mo. Their BMI was 22.8 + −3.4 kg/m2. The pts were arbitrarily divided in two groups according to their s.albumin (alb) levels. Group I (GrI): 24 pts with s.alb < 4 g/dl (13 on CBM 4 g/dl (8 on CBM and 8 on ≥ and 11 on SPM), Group II (GrII): 16 pts with s.alb SPM).

Results: No differences were observed between the two groups regarding: age, sex, BMI, time on HD, UFV, Hct, s.creatinine, glucose, Chol, TG, HDL, LDL, Apo-A1, Apo-B TNF-a, IL-6, Ca x P product, smoking, hypertension, coronary artery disease.

The s. levels of Lp(a), FNG, iPTH, IL-8 and leptin were measured before a single HD. IL-8 and leptin values were determined using specific immunoassays Quantikine®, Quantikine® human leptin respectively.

Results:

HD	iPTH (pg/mL)		Lp (a)mg/dl		FNG (mg/dl)		IL-8 (pg/mL)		Leptin (ng/mL)	
	CBM	SPM	CMB	SPM	CBM	SPM	CBM	SPM	CBM	SPM
Cr I	484 ± 135	110 ± 32*	42 ± 43	29.7 ± 17	356 ± 68	378 ± 124*	19 ± 8	17.5 ± 11	13.7 ± 285	22.6 ± 25*
Cr II	533 ± 97	364 ± 108	47.6 ± 58	29.5 ± 27	453 ± 113	360 ± 99	17 ± 4	32.4 ± 19	27.6 ± 36	4.4 ± 6.6

* p < 0.05, Lp(a) normal values (n.v); <30 mg/dl, FNG n.v: 150–400 mg/dl.

Conclusion: a) SPM influence favourably iPTH levels in pts with s.alb < 4 g/dl (malnourished), b) Lp(a) levels are not influenced by either type of HD independently of s.alb levels, c) CBM reduces more the FNG than the SPM in pts with s.alb < ≥4 (malnourished), d)CBM reduce IL-8 levels in pts with s.alb (well-nourished).

W-PO40010

RISK FACTORS ASSOCIATED WITH CORONARY ARTERY DISEASE IN HEMODIALYSIS PATIENTS

<u>LYGERI PAULOS SOUBASSI</u>[1], EMMANUEL PAPADAKIS[1], GEORGE POULOS[1], ALEKSANDROS KOUVELIS[1], STYLIANOS NIKOLAOS ZEREFOS[1], THEODOROS CHIRAS[1], SIMEON KAPNISIS[1], IOANNIS TSAPAKIDIS[1], GRIGORIOS DAGLAS[1], DIMITRIOS VALIS[1], NIKOLAOS STYLIANOS ZEREFOS[1]

Renal Unit, Hygeia Hospital, Greece[1]

Aim: to determine the prevalence of coronary heart disease (CAD) in different age groups of pts on chronic hemodialysis (HD), and its association with the presence of predisposing factors. 171 dialysis pts were included (107 M and 64 F). Mean age of pts was 67 ± 13 years, mean time on dialysis 52.7 ± 44 mo and BMI 25.9 ± 3.7 Kg/m2. Fifty pts (29.2%) were clinically diagnosed with CAD based on angiography or thalium excersise. They were evaluated for age, sex, smoking, hypertension, obesity, diabetes mellitus (DM), hyperlipidemia, anemia, low albumin levels, secondary hyperparathyroidism (SHP), chronic inflammation, as evidenced by elevated levels of CRP and hyperhomocysteinemia (HOC). Fifty two (30.4%) were hypertensive, 27 (15.8%) were smokers, 47 (27.5%) were diabetic, 76 (44.4%) had hyperlipidemia, and 66 (38.6%) had SHP. The incidence of CAD according to age group was: 20–29 yo (2 pts) – no CAD, 30–39 yo (9 pts) – no CAD, 40–49 yo (29 pts) – 1 patient with CAD, 50–59 yo (32 pts) – 7 pts with CAD, 60–69 yo (38 pts) – 12 pts with CAD, 70–79 yo (47 pts) – 21 with CAD, >80 yo (14 pts) – 9 with CAD. There was a statistically significant association of increasing age and CAD (p < 0.0001). Relative risk (RR) was significantly increased in 1) M pts compared to F pts (RR: 8.56, p < 0.001), 2) anemic pts compared to pts with hemoglobin levels >11 g/dl (RR: 8.26, p < 0.0001), 3) obese pts compared to pts with BMI ≥ 30 (RR: 5.09, p < 0.005), 4) pts with HOC compared to pts with levels of homocysteine <15 μM (RR: 4.14, p < 0.0001) and 5) pts on dialysis ≥36 mo compared to pts on dialysis ≤12 mo (RR: 1.3, p < 0.0001). The prevalence of CAD in dialysis pts is significantly increased with age, male sex, obesity, time on HD, anemia and HOC.

W-PO40011

B-TYPE NATRIURETIC PEPTIDE (BNP) AND AMINO-TERMINAL PROBNP IN PATIENTS WITH CHRONIC KIDNEY DISEASE: RELATIONSHIP TO RENAL FUNCTION AND LEFT VENTRICULAR HYPERTROPHY

<u>NASIR ABBAS</u>[1], SUSAN VICKERY[2], IAN JOHN[1], MICHELLE WEBB[1], CHRISTOPHER PRICE[3], MICHELLE KEMPSON[2], EDMUND LAMB[2]

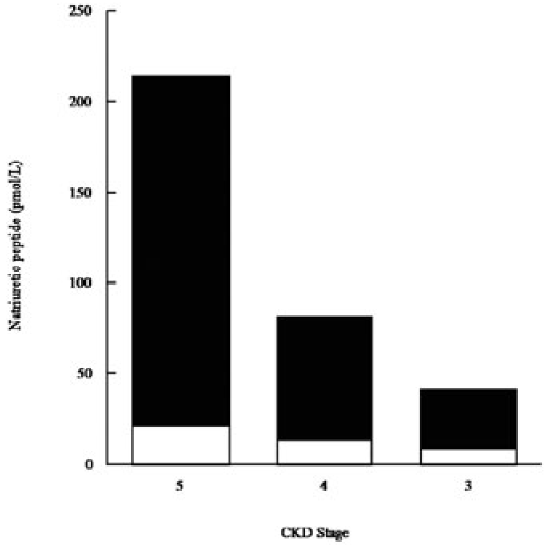
Department of Renal Medicine, Kent and Canterbury Hospital, Canterbury, UK[1], *Department of Biochemistry, Kent and Canterbury Hospital, Canterbury, UK*[2], *Global Clinical Research Diagnostic Division, Bayer Health Care, Newbury, Berkshire, UK*[3]

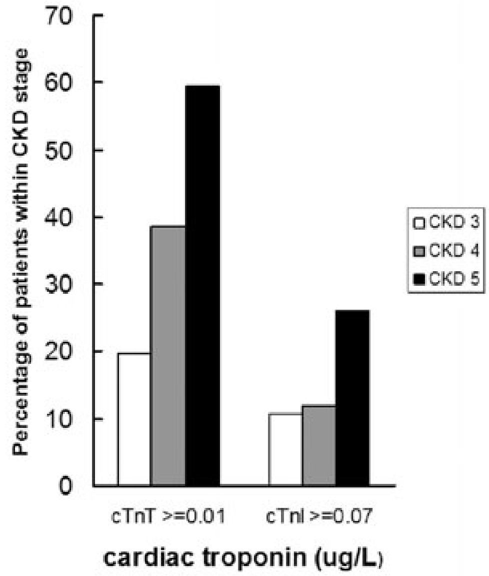
Background: Most patients with progressive chronic kidney disease (CKD) develop cardiovascular complications. Natriuretic peptides are novel markers that can be used to predict and monitor heart failure, but the effect of renal disease on these markers is not fully understood. The present study explored circulating B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) concentrations and their relationships to clinical variables in a cohort of patients with CKD.

Methods: Plasma BNP (Bayer Diagnostics plc) and NT-proBNP (Roche Diagnostics Ltd) concentrations were measured in 213 patients, of which 55 had stage 3 (median glomerular filtration rate [GFR] 39.0 mL/min/1.73 m^2, median age 68.0 y, median left ventricular mass index [LVMI, g/m^2] 126.9, 42 males, 11 diabetics), 66 stage 4 (median GFR 22.0 mL/min/1.73 m^2, median age 71.0 y, median LVMI 132.2, 45 males, 24 diabetics) and 92 stage 5 (median GFR 9.5 mL/min/1.73 m^2, median age 65.5 y, median LVMI 155.3, 50 males, 22 diabetics) CKD. Left ventricular hypertrophy (LVH) was considered present if the LVMI exceeded 125 g/m^2.

Results: Plasma BNP and plasma NT-proBNP concentrations increased with declining glomerular filtration rate (GFR, $p < 0.0001$). GFR had an independent effect on plasma BNP and, to a greater extent, plasma NT-proBNP concentration: mean BNP concentration increased by 20.7 % per 10 mL/min/1.73 m^2 reduction in GFR compared to 40.7% for NT-proBNP. The NT-proBNP/BNP ratio increased with CKD stage ($p < 0.0001$). Median plasma BNP and plasma NT-proBNP concentrations were higher in patients with LVH within each CKD stage.

Conclusion: GFR and LVH have independent effects on both plasma BNP and plasma NT-proBNP concentrations in patients with CKD. NT-proBNP would appear to be more affected by declining kidney function in keeping with the hypothesis that its clearance is predominantly renal. Our data have significant implications for the use of these cardiac biomarkers in patients with CKD.





W-PO40012

CARDIAC TROPONINS AND RENAL FUNCTION IN PATIENTS WITH CHRONIC KIDNEY DISEASE: RELATIONSHIP TO LEFT VENTRICULAR HYPERTROPHY

NASIR ABBAS[1], MICHELLE WEBB[1], IAN JOHN[1], MICHELLE KEMPSON[2], AISLING POTTER[2], CHRISTOPHER PRICE[3], SUSAN VICKERY[2], EDMUND LAMB[2]

Department of Renal Medicine, Kent and Canterbury Hospital, Canterbury, UK[1], Department of Biochemistry, Kent and Canterbury Hospital, Canterbury, UK[2], Bayer Diagnostics plc, Newbury, Berkshire, UK[3]

Background: Serum cardiac troponin (cTn) concentrations are commonly increased in end-stage renal disease (ESRD) in the absence of an acute coronary syndrome, with the data on cardiac troponin I (cTnI) being more variable than that for cardiac troponin T (cTnT). There is little information on cTn concentrations in patients with chronic kidney disease (CKD) who have not commenced dialysis.

Methods: We studied 222 patients, of which 56 had stage 3 (median glomerular filtration rate [GFR] 39.0 mL/min/1.73 m^2, median age 68.0 y, median left ventricular mass index [LVMI, g/m^2] 126.8, 43 males, 11 diabetics), 70 stage 4 (median GFR 22.0 mL/min/1.73 m^2, median age 71.0 y, median LVMI 142.1, 49 males, 25 diabetics) and 96 stage 5 (median GFR 9.0 mL/min/1.73 m^2, median age 64.5 y, median LVMI 154.5, 53 males, 23 diabetics) CKD. Left ventricular hypertrophy (LVH) was considered present if the LVMI was >125 g/m^2.

Results: Overall, serum cTnT (Roche Diagnostics Ltd) concentration was increased above the 99th percentile upper limit of normal in 43% of CKD patients, compared to 18% for cTnI (Bayer Diagnostics plc). Serum cTnT/cTnI concentrations were more likely to be increased as CKD progressed, being elevated respectively in 11/6 stage 3, 27/8 stage 4 and 57/24 stage 5 patients ($p < 0.0001/p < 0.02$). Amongst the 38 patients with detectable cTnI, 32 had detectable cTnT (r_s 0.67, $p < 0.0001$). Increased cardiac troponin concentrations were associated with the presence of left ventricular hypertrophy ($p < 0.01$).

Conclusions: The prevalence of elevated cTn concentrations in CKD patients is not vastly different to that observed amongst dialysis patients, and increases may occur early in CKD. It seems likely that these observations relate to the concomitant but independent presence of both failing renal function and left ventricular hypertrophy. These data have important implications for the management of patients with pre-ESRD CKD presenting with chest pain.

W-PO40013

FACTORS INFLUENCING PULSE PRESSURE IN DIALYSIS POPULATION

SUNITA BAVANANDAN[1], ANITA DAS[2], SHARON CHEN[2], SANJAY RAMPAL[2], T O LIM[2], ZAKI MORAD[1]

Dept of Nephrology, Hospital Kuala Lumpur[1], Clinical Research Center, Hospital Kuala Lumpur[2]

Background: The excess cardiac risk associated with end-stage renal disease may be partly mediated by increased arterial stiffness, for which pulse pressure (PP) is a surrogate marker. Secondary hyperparathyroidism and abnormalities of calcium-phosphate metabolism may be among the factors contributing to the increased arterial stiffness. Hence, a cross-sectional study was carried out to assess the relationship between PP and various demographic, physiological and laboratory parameters including markers of secondary hyperparathyroidism.

Methods: Statistical analysis was performed for data captured in the local renal registry for 12.447 adult patients at onset of end-stage renal disease from 1st January 1993 to 31st December 2002. Multiple linear regression analysis was used to test for associations between PP and various parameters including age, gender, time on dialysis, pre-dialysis systolic and diastolic blood pressure (SBP, DBP), serum calcium, se phosphate, serum intact parathyroid hormone (se iPTH), serum total cholesterol and haemoglobin.

Results: The final model for multivariate linear regression analysis identified age, diabetes mellitus, requirement for anti-hypertensive treatment, corrected serum calcium and dialysis modality as variables significantly affecting PP. For corrected serum calcium levels equal or above 2.6 mmol/L, every 1 mmol/L of serum calcium above 2.6 was associated with a 1.218 mmHg increment in PP ($p = 0.011$, confidence interval 0.27 to 2.16). Other parameters of secondary hyperparathyroidism such as serum phosphate and serum iPTH had a positive association with PP but did not reach statistical significance.

Conclusion: Older age, diabetes, the need for anti-hypertensive treatment, elevated corrected serum calcium and haemodialysis are positively associated with a higher PP in the dialysis population. Secondary hyperparathyroidism and abnormalities of calcium-phosphate metabolism may have an impact on PP but further studies are required to clarify this.

W-PO40014

HYPERHOMOCYSTEINEMIA THERAPY IN HEMODIALYSIS PATIENTS: FOLINIC VERSUS FOLIC ACID IN COMBINATION WITH VITAMIN B6 AND B12

MITRA MAHDAVI MAZDEH[1], ROZITA ABOLGHASEMI[2], MOHAMADREZA KHATAMI[1], MAHBOOB LESSAN_PEZESHKI[1], FAROKHLAGHA AHMADI[1], SEPIDEH SEIFI[1], SIMA MAZIAR[1]
Faculty Of Medicine_TUMS[1], Department Of Nephrology[2]

It has been reported that serum homocysteine concentrations are elevated in hemodialysed patients. Role of hyperhomocysteinemia in atherothrombogenic process provoked general attempts of any therapeutic protocol to control it. Clinical data do not support any effect of low dose folic acid (1–5 mg/d) in decreasing homocysteine level in hemodialysis patients in contrast to general population. These data have shown that hemodialysis patients have resistance to the action of folic acid. On the other hand, it seems that reduced form of folate, ie methyltetrahydrofolate or folinic acid used by some has led to a more efficient and significant decrease of plasma homocysteine concentration than folic acid. Some suggested that the tHcy lowering effect of high dose oral folinic acid or oral high dose of folic acid were comparable.
To clarify the findings better, we conducted a randomized controlled trial aimed at evaluating whether oral folinic acid provided improved tHcy-lowering efficacy in hemodialysis patients compared with oral folic acid.
Method: In a 7-month prospective, randomized controlled trial. 30 chronic hemodialysis patients, matched for age, gender, dialysis duration and average screening pre-treatment-fasting tHcy levels, were given either 45/week of oral calcium folinate or 45/week oral folic acid. All pateints received 300 mg/week IV VIT B6 and 3 mg/week of oral vitamin B12.
Results: Fasting tHcy decreased singnificantly but to different extent in both groups after 1 month of treatment, but at 2 month of treatment the decrease was less significant. Mean percentage of tHcy reduction at one month in folinic acid group and in folic acid group was 26% and 41% respectively. Mean percentage of tHcy reduction at two months in folinic acid and folic acid groups were 15% and 20% respectively.
Conclusion: These findings show that the tHcy-lowering effects of high dose folic acid was greater than the effect of high dose folinic acid.

W-PO40015

DETERMINANTS OF EARLY-ONSET ATHEROSCLEROSIS IN PATIENTS ON HEMODIALYSIS: OVER THE COURSE OF ONE YEAR

AYSEGUL ZUMRUTDAL[1], SENOL DEMIRCAN[2], MEHMET BALTALI[2], METIN SINGAN[1], SIREN SEZER[1], FATMA NURHAN OZDEMIR[1], MEHMET HABERAL[3]
Baskent University Faculty of Medicine, Department of Nephrology[1], Baskent University Faculty of Medicine, Department of Cardiology[2], Baskent University Faculty of Medicine, Department of General Surgery[3]

The aim of this prospective study was to assess the determinants of the progression of carotid artery intimae-media thickness (C-IMT) over one year in patients on regular HD therapy with no clinical evidence of atherosclerosis.
Originally, a cohort of 72 HD patients (43 men, 29 women; mean age, 34.5 ± 10.6 years, aged 18 to 55; mean time on HD, 47.9 ± 40.0 months) participated in the study. Fifty-four patients (29 men, 25 women, mean age 33.3 ± 10 years, and aged 17 to 55, mean time on HD 49.4 ± 43 months) completed the one year period of the study. To assess the determinants of C-IMT progression, hematocrit (Hct)-corrected erythrocyte sedimentation rate (ESR), beta-2 microglobulin (β2M), serum cardiac troponin I (cTnI), C-reactive protein (CRP), lipid profile, fibrinogen and homocysteine levels were assessed at baseline and after 12 months. The mean C-IMT at baseline, 0.59 ± 0.06 mm, increased by a mean of 0.05 ± 0.05 to 0.64 ± 0.07 mm ($p < 0.001$) in one year. At the end of follow-up: 41 patients (75.9%) had increase in C-IMT, 13 patients remained stable (min –0.02 max 0.17 mm). In 72 patients, baseline C-IMT was independently related with age ($r = 0.015$, $p = 0.02$), β2M ($r = 0.015$, $p = 0.001$), and log-transformed CRP ($r = 0.011$, $p = 0.03$) in linear regression analysis. In 54 patients, C-IMT progression was correlated with age ($r = 0.406$, $p = 0.02$), fibrinogen ($r = 0.301$, $p = 0.027$) and increase in CRP ($r = 0.585$, $p < 0.001$). Linear regression analysis revealed that age ($\beta = 0.282$, $p < 0.013$) and increase in CRP levels ($\beta = 0.570$, $p < 0.001$) were associated with increases in C-IMT independently (R^2 for the model 0.146).
Our results suggest that beyond age, increases in CRP levels over one year play a significant role in the progression of C-IMT in HD patients without any clinical evidence of atherosclerosis.

W-PO40016

ROLE OF INTRA-QRS POTENTIALS ANALYSIS TECHNIQUE IN FREQUENCY DOMAIN SIGNAL-AVERAGED ELECTROCARDIOGRAPHY FOR IMPROVING VENTRICULAR ARRHYTHMIA DETECTION IN CHRONIC RENAL FAILURE HEMODIALYSIS PATIENTS IN TAIWAN

ING–FANG YANG[1], CHUN-CHENG LIN, CHIH-MING CHEN[2], TEN-FANG YANG[3]
Jen-Chi General Hospital[1], Department of Electric Engineering, National Taiwan University of Science and Technology[2], Graduate Institute of Medical Informatics and Department of Cardiology, Taipei Medical University and Hospital[3]

The unpredictable characteristics of abnormal intra-QRS potential (AIQP) component originated from the QRS complex in signal-averaged electrocardiogram (SAECG) have been proposed as an index of risk stratification for ventricular arrhythmias (VA). The aim of the present study is to evaluate the role of AIQP for the detection of VA in frequency domain SAECG in Taiwanese chronic renal failure (CRF) patients under maintenance hemodialysis (HD) therapy.
There were 155 normal controls (N) and 36 CRF patients recruited. The AIQP was estimated from the low amplitude modeling residual of QRS complex after a discrete cosine transformation in the three orthogonal SAECG X, Y, and Z leads. A locally developed parametric AIQP model was used to compare with filtered QRS duration (fQRS), root mean square voltage in last 40 ms of the QRS intervals (RMS_{40}) and duration of low amplitude signals <40 μV (LAS_{40}) calculated from time domain SAECG via the cross-correlation coefficient function. The mean AIQP in leads X and Z in CRF patients are statistically ($p < 0.05$) greater than N either pre or post HD, while mean AIQP in lead Y is smaller than N pre HD ($p < 0.006$). There is a 3 ms increase ($p < 0.0007$) of mean fQRS post HD. There is no statistically significant change in RMS_{40} or LAS_{40}. In 92% of CRF patients, there is a significantly greater mean AIQP in all three leads. There is also an increase of fQRS in 75% patients post HD.
Either fQRS in time domain or AIQP in frequency domain SAECG differed significantly post HD in CRF patients than N. This coincides with the fact that higher incidence of VA post HD. It is demonstrated that higher proportion alteration of AIQP in all three leads can improve the detection of VA.

	AIQPX(μV)	AIQPY(μV)	AIQPZ(μV)	fQRS(ms)
N	4.6 ± 1.6	6.1 ± 2.3	6.1 ± 3.0	90.4 ± 8.6
Pre HD	5.9 ± 2.8	5.4 ± 2.5	9.4 ± 4.1	91.5 ± 10.8
Post HD	7.0 ± 3.5	6.5 ± 2.7	10.3 ± 4.9	94.5 ± 11.1

W-PO40017

EFFECT OF LOVASTATIN ON C-REACTIVE PROTEIN AND HEMOGLOBIN IN HEMODIALYSIS PATIENTS

FARROKH LAGHA AHMADI[1], KHADIJEH ESLAMI[1], SIMA.MAZIAR[2], M.LESSAN PEZESHKI[1], MOHAMAD REZA.KHATAMI[1], MITRA.MAHDAVI MAZDEH[1], SEPIDEH.SEIFI[1], EFFAT.RAZEGHI[1]
Facuality of Medicine-Tums[1], Facuality of Medicine[2]

C-reactive protein whose synthesis in the liver is a markers of an acute phase reaction. In hemodialysis
Patients high CRP level are also associated with lower hemoglobin levels resistant to EPO. Recently statin
Have shown anti inflammatory properties in addition to their lipid lowering effect. We designed a 3 month Prospective randomized control study to assess the safety and efficacy of lovastatin in reduction serum CRP level and increment Hb. Patients presenting with illnesses and or use of drugs that may affect CRP Level excluded. We evaluated 27 chronic hemodialysis patients with CRP ≥ 10 mg/l, (20 men and 7 women).
After randomization, group A included 14 patients treated with lovastatin 20 mg/day orally and group B included 13 patients without any medication. Qualitative/quantitative parameters were homogeneous between groups at baseline. After 3 month we evaluated Hb and CRP in two groups in group A median CRP level decreased from 18.9(10.5–72.8)mg/l at baseline to 10(1.8–56.3)mg/l after

3 month p = 0.05 in group B, values were 21(10.1–79.2)mg/l at baseline and 32(0.1–92.1)mg/l after 3 month p = 0.7 mean. Hb level in group A increased from 9.5 ± 2 to 10.5 ± 1.4 mg/dl (p = 0.01) in group B values were 9 ± 1.8 at baseline and 10 ± 1.7 after 3 month, (p = 0.01).
Conclusion: adminstration of lovastatin is safe in patients on long-term HD therapy. In addition has beneficial effect on CRP level, but did not different in hemoglobin between two groups.

W-PO40018

TOE-BRACHIAL INDEX (TBI) AND MACROPHAGE COLONY STIMULATING FACTOR (MCSF) IN HD PATIENTS

HIRONOBU INOUE[1], KAZUTAKA HARAGUCHI[1], MASASHI KUBO[2], KIICHIRO TAGO[2]. MITSURU TAKAHASHI[1], TAKEHIRO SAITO[1], HIROKI SHIMURA[1], TETSURO KOBAYASHI[1]
3rd Department of Internal Medicine, Yamanashi University[1], Tago Kidney-Urology Clinic[2]

Purpose: Cardiovascular diseases are the leading causes of death in chronic hemodialysis (HD) patients. Pulse wave velocity (PWV), aortic calcification index (ACI) measured by CT, and carotid intima-media thickness (IMT) measurement by ultrasonography are the atherosclerotic parameters available at outpatient settings. TBI was newly added to the members of the above parameters and has been reported to have close relationship to the patients' long-term outcome and QOL.
Patients and Methods: In the present study, we measured TBI in 145 (male 100, female 45) chronic HD patients and compared it to other atheroscleortic parameters. Proinflammatory cytokines (IL-6, high sensitive CRP, and macrophage colony stimulating factor (MCSF)) were measured by commercially available kits.
Results: Fourteen patients had TBI 0.6, while only 7 out of 14 had experienced intermittent claudication or coldness of the extremities. Existence of low TBI was associated with decreased Anckle-Brachial Index (ABI) but not with elevated baPWV and ACI. TBI was not associated with serum albumin, HDL, TChol, Ca × P, WBC, and RBC levels. Among the inflammatory cytokines tested, only MCSF was associated with TBI. Backward regression analysis of the data revealed that smoking (p = 0.004), CTR (%) (0.015), body mass index (BMI) (0.029), MCSF (0.032), and serum total protein levels (0.041) were the only independent variable that were related to TBI. Regression analysis of the data also revealed that MCSF was a strong independent variable that predicted the existence of abnormal ABI, increased ACI, and increased carotid IMT. hsCRP was a significant variable in carotid IMT, but not in other atheroscleortic parameters.
Conclusion: Our present study suggests that MCSF plays an important role in the progression of cardiovascular disease in HD patients.

W-PO40019

BRAIN NATRIURETIC PEPTIDE AS A CARDIOVASCULAR RISK MARKER IN HIGH FLUX DIALYSED PATIENTS

P. MATIAS[1], I. AIRES[1], C. JORGE[1], S. MARUM[2], H. LAGE[3], C. GIL[1], F. GOMES[1], E. ARRANHADO[3], A. FERREIRA[1]
Hemodial, Vila Franca de Xira, Portugal[1], Intensive Care Unit, Hospital Curry Cabral, Lisbon, Portugal[2], Laboratory of Immunology, Hospital Curry Cabral, Lisbon, Portugal[3]

In order to analyse the value of brain natriuretic peptide (BNP) serum levels as a marker of cardiovascular (CV) disease, we performed a prospective study in which we evaluated: a) pre and post hemodialysis (HD) BNP values, b) the effects of feedback control of relative blood volume (BV) on the hemodynamic stability and on BNP variation and c) BNP correlation with other known CV risk factors. Clinical data included cause of CRD, presence of diabetes, hypertension, coronary artery disease (CAD) and cardiac insufficiency (CI). In each mid week HD session (without BV monitor (BVM) = norm and with BVM), episodes of hypotension, systolic, diastolic, mean blood and pulse pressures (PP) were evaluated. Laboratorial data included: C-reactive protein, albumin, iPTH, BNP and Kt/v. Echocardiographic evaluation (M mode, bidimensional and Doppler) was performed in the same conditions.
We studied 48 random pts with mean age of (±SD) 70 ± 13.9 years, 62.5% female and 43.8% diabetic, with mean HD time of 38.1 ± 29.3 months and all dialysed with high flux helixone filters.
We found no variation on mean ultrafiltration volume, mean systolic or diastolic blood pressure and effective HD time between those two modality HD sessions. BNP (pre and post HD) was not different between these 2 sessions nor did it change significantly during the HD procedure (BNP pre HD norm = 0.36 ± 0.15 ng/ml, BNP pre HD BVM = 0.39 ± 0.17 ng/ml, BNP post HD norm = 0.37 ± 0.17 ng/ml, BNP post HD BVM = 0.39 ± 0.17 ng/ml).
On univariate analysis, the presence of CI was significantly correlated with BNP (r = 0.29, p = 0.04). On multivariate analysis, the presence of a high BNP (percentile 75) was a positive predictive factor of higher values of PP (p = 0.02, Exp(B) = 1.07).
In this group of pts higher BNP serum levels were a good marker of CV risk. High flux dialysis (with or without BVM) did not induce variation of serum BNP levels.

W-PO40020

CORRELATION BETWEEN REGIONAL ADIPOSE TISSUE DISTRIBUTION AND INSULIN RESISTANCE IN NONDIABETIC HEMODIALYSIS PATIENTS

HYEONG CHEON PARK[1], SHIN WOOK KANG[1], KYU HUN CHOI[1], SUNG KYU HA[1], HO YUNG LEE[1], DAE SUK HAN[1], CHUL WOO AHN[1]
Yonsei University College of Medicine, Department of Internal Medicine, Korea[1]

Visceral adipose tissue is well recognized to be significantly related to insulin resistance and excessive visceral fat accumulation in hemodialysis patients has been reported. Recently the role of intramuscular lipid components (measured as low-density muscle area) in insulin resistance has been suggested in type 2 diabetic patients. However, such this relation has not been investigated in hemodialysis patients. We examined the link between regional adipose tissue distribution (low-density muscle of midthigh and visceral abdominal fat aggregation) and insulin resistance in nondiabetic chronic hemodialysis patients. Abdominal visceral fat area (AVFA), abdominal subcutaneous fat area (ASFA) and low-density muscle area (LDMA, 0 to +30 Hounsfield units) of midthigh were measured by computed tomography (Tomoscan 350, Philips, Mahway, NJ). Whole body fat mass and fat-free mass was assessed by bioimpedance analysis (Body fat analyzer, Cas Co, Seoul, Korea) and the homeostasis model assessment (HOMA) score was calculated to assess whole body insulin sensitivity.
Fourteen clinically stable nondiabetic hemodialysis patients (6 male and 8 female, mean age 64.2 ± 14.2 years) were recruited for the study. Body mass index was identical in both sexes, however, the percentage of total body fat was higher in women (28.3 ± 5.5% vs 23.7 ± 5.8%, p < 0.05) and the percentage of fat-free mass was higher in men (p < 0.05). The mean values of AVFA, ASFA and LDMA were 106.4 ± 64.8, 115.8 ± 74.5, and 21.2 ± 10.4 cm^2, respectively. Fasting serum insulin levels were significantly related to ASFA (r = 0.792, p = 0.006) and LDMA (r = 0.749, p = 0.013). HOMA score correlated significantly with AVFA (r = 0.603, p = 0.05), ASFA (r = 0.739, p = 0.015) and LDMA (r = 0.761, p = 0.011). Our results suggests that low density muscle of midthigh, which accounts for a relatively small portion of the total skeletal muscle, seems to be a potential marker of insulin resistance in nondiabetic hemodialysis patients.

W-PO40021

EFFECT OF LOW DENSITY LIPOPROTEIN RECEPTOR GENE POLIMORPHISM ON SERUM LIPID LEVEL IN HEMODIALYSIS PATIENTS

YUXIN WANG[1], HEQUN ZOU[1], QINGQIN LI[2]
Dept. Nephrology, The 5th Affiliated Hospital of Sun Yat-sen University, China[1], Laboratory, The 5th Affiliated Hospital of Sun Yat-sen University, China[2]

Background: Dislipidemia is one of the main reasons resulting in cardiovascular disease in hemodialysis patients, but the mechanism is not known clearly. In this study, low density lipoprotein receptor (LDLR) gene polymorphism was determined to investigate the effect on serum lipid levels in hemodialysis patients.
Method: Serum total cholesterol, triglyceride, low density lipoprotein, high density lipoprotein cholesterol, apolipoproteins A1, B, E and lipoprotein(a) were measured, and polymerase chain reaction-restriction fragment length polymophism was used to detect gene polymorphism of LDLR intron 4 and exon 13 in hemodialysis patients.
Results: The levels of serum triglyceride and apolipoprotein B were significantly higher than those in controls. The data of LDLR intron 4 Taq I and LDLR exon 13 Ava II gene polymorphism detected by polymerase chain reaction-restriction fragment length polymophism showed neither polymorphism of LDLR intron 4 Taq I nor LDLR exon 13 Ava II was significantly different between controls and

hemodialysis patients. The effect of LDLR gene polymorphism on serum lipid levels was different in controls and hemodialysis patients. LDLR intron 4 Taq I genotypes were associated with serum total cholesterol level of controls, but with serum triglyceride level of hemodialysis patients in the following order: –/–, +/– and +/+ ($P < 0.05$ and $P < 0.01$ respectively). LDLR exon 13 Ava II genotypes were associated with serum triglyceride level in controls in the following oder: –/–, +/– and +/+ ($P < 0.05$), but there was no significant difference of any lipid levels in hemodialysis patients with different genotypes of LDLR exon.
Conclusion: Hemodialysis patients were liable to develop hypertriglyceridemia and hypocholesteroldemia. Taq I gene polymorphism in LDLR intron 4 could affect the activity of LDLR and TG level in hemodialysis patients, the patients with genotype Taq I –/– in LDLR intron 4 were liable to develop dislipidemia.

W-PO40022

EFFECT OF HEMODIALYSIS DOSE AND FLUX ON LIPOPROTEINS (LP) IN THE HEMO STUDY

MADHUKAR CHELAMCHARLA[1], GUOFEN YAN[2], MICHAEL ROCCO[3], GERALD SCHULMAN[4], BRENDAN TEEHAN[5], ROBERT TOTO[6], ALFRED K. CHEUNG[1]
University of Utah, Salt Lake City, UT, USA[1], Cleveland Clinic Foundation, Cleveland, OH, USA[2], Wake Forest University, Winston-Salem, NC, USA[3], Vanderbilt University, Nashville, TN[4], Lankenau Hospital, Wynnewood, PA[5], University of Texas Southwestern Medical Center, Dallas, TX, USA[6]

Background: Hemodialysis (HD) patients have high rates of cardiovascular mortality. The role of various LP in the pathogenesis of cardiovascular disease in this population and the effect of HD dose and flux on plasma LP are unclear.
Methods: An ancillary study of the HEMO Study was performed in 253 patients. Predialysis plasma samples were collected at baseline and 3 months after randomization to either high or standard dose (urea eKt/V of 1.45 vs. 1.05 respectively) and either high-flux or low-flux HD (β2M clearance of >20 ml/min vs. <10 ml/min respectively). The data were analyzed for (a) the effect of dose and flux on changes in plasma LP levels using 2-sample t-tests and (b) the association between baseline LP levels and all-cause mortality using multivariable Cox regression models, adjusting for demographics and co-morbidities.
Results: The percent change in LP levels from baseline to 3 months was:

	High Kt/V	Std Kt/V	High flux	Low flux
LDL-C	+3.9%	+2.0%	+1.3%	+4.7%
HDL-C	+2.6%	–0.9%	–0.9%	+4.5%
ApoAI	–2.5%	–4.3%*	–4.1%*	–2.7%
ApoB	–3.6%	–0.6%	+4.8%	–1.8%
Lp(a)	+54.0%*	+47.2%**	+60.0%*	+39.9%**

* $p < 0.05$ and ** $p < 0.001$ vs. baseline.

There were no statistically significant differences in the longitudinal change in LP levels between high dose and standard dose and between high flux and low flux. Cox analysis showed an association between increased mortality and both higher plasma HDL cholesterol (RR = 1.60 [1.03–2.46] for each 10 mg/dL increase; $p = 0.03$) and apoB levels (RR = 1.37 [1.10–1.71] for each 10 mg/dL increase; $p = 0.005$). No association was seen with LDL cholesterol, apoAI and Lp(a).
Conclusion: Our randomized trial results showed that in HD patients with a 3-month follow-up, there were no major changes in plasma LP levels except for an increase in Lp(a), which was observed regardless of the dialysis dose and flux. Paradoxically, higher HDL cholesterol but not Lp(a) level was associated with increased long-term all-cause mortality. The role of LP in the pathogenesis of cardiovascular disease in HD patients has yet to be defined.

W-PO40023

IMPACT OF NANDROLONE ON LIPID PROFILE IN HEMODIALYSIS PATIENTS

KHADIDJE MAKHDOOMI[1], ZAHRA YEKTA[1], ALI GHAFARI[1], PEDRAM AHMADPOOR[1], ANVAR RAVANDI[1], ALIREZA AHMADIZAD[1], MOHAMAD HOSSEINI ARABLOO[1]
Uromieh University Of Medical Sciences[1]

Background: Cardiovascular disease is the most common cause of death in Hemodialysis patients.
Lp(a) is an independent risk factor for atherosclerosis and in many studies elevated plasma levels of Lp(a) was shown in hemodialysis patients. The aim of this study was to evaluate the impact of Nandrolone Decanoate (used as an adjunct therapy for treatment of anemia) on plasma Lp(a) in hemodialysis patients.
Material and Methods: In a clinical trial, hemodialysis patients were randomly allocated in 2 groups, each consists of 36 patients.
Group 1 was treated with 100 mg intramuscular injections of Nandrolone Decanoate weekly for 6 months and Group 2 was followed as control.
Plasma concentrations of cholesterol, triglycerides, albumin, LDL, HDL, Apo A1, Apo B, and Lp(a) were measured before the injection and 2, 4, 6 and 9 months after injections in both groups. Repeated measured ANOVA was used.
Results: Mean of plasma Lp(a) concentrations in both groups in given months are shown in table

groups	month: zero	Mo: 2	Mo: 4	Mo: 6	Mo: 9
Group1	48.8 ± 21.7	43.4 ± 19.8	39.4 ± 11.9	64.3 ± 28.2	64.3 ± 30.9
Group2	56.1 ± 31	57.6 ± 27.4	48.2 ± 26.3	76 ± 37.4	67 ± 31.1

There was no statistically significant differences in terms of Apo B, TG, HDL, Lp(a) and albumin in both groups but the level of total cholestrol, LDL, Apo A1 were decreased in group 1 that was statistically significant ($P = 0.05$).
Conclusion: In our study we could not find any influence of nandrolone on Lp(a) and the impact of nandrolone in decrement of total cholestrol and LDL must be balanced against decrement of Apo A1. For better clarifications further studies will be helpful.

W-PO40024

INTRACELLULAR FREE CHOLESTEROL ACCUMULATION IN ER TRIGGERS APOPTOSIS IN MACROPHAGES AND HUMAN VASCULAR SMOOTH MUSCLE CELLS

XIONG ZHONG RUAN[1], JIANLING TAO[1], JOHN F. MOORHEAD[1], STEPHEN H. POWIS[1], ZAC VARGHESE[1]
Royal Free and UC Medical School[1]

Cardiovascular complications caused in part by atherosclerotic disease represent the largest single cause of mortality in dialysis patients with chronic kidney disease. Macrophages and vascular smooth muscle cells (VSMCs) play an important role in the atherosclerotic process through foam cell formation. Apoptosis is detectable in fatty streaks and is more abundant in advanced plaques. Using cells of the human THP-1 macrophages and VSMCs, we investigated the role of four different lipids (LDL, Ac-LDL, free cholesterol and 25-hydroxycholesterol) in the apoptotic process. Cell proliferation was examined using ^{3}H incorporation; cell apoptosis was analysed by DAPI, Annexin-V stain, and caspase-3,-7/viability assay. Intracellular free/total cholesterol was measured using an enzymatic method. Endoplasmic reticulum (ER) sterol pool was analysed TLC using [1-^{14}C] oleoyl-Coenzyme A.
Results demonstrated that (i) cell apoptotic process was cell type and lipid type dependent. Native LDL and free cholesterol did not induce apoptosis in these cell types. Ac-LDL (50–100 mg/ml) and 25-hydroxycholesterol (15 mg/ml) inhibited cell proliferation and induced apoptosis in macrophages and VSMC. Interestingly, the apoptosis effect was enhanced by administration of ACAT inhibitor FR179254 (800 nM) or 58035 (10 mg/ml) which increases free cholesterol accumulation in ER by blocking free cholesterol esterification; (ii) surprisingly; there was no significant total free cholesterol accumulation in macrophages and VSMCs after incubation with lipids, suggesting total intracellular free cholesterol is regulated to prevent accumulation; (iii) furthermore, free cholesterol content in ER was significantly increased after cholesterol loading and associated with apoptotic rate in macrophages and VSMCs.
These data suggest that the change of total intracellular free cholesterol is slow to response and not a good marker for predicting cell cytotoxicity. However, ER cholesterol pool which just contains up to 4% of total cholesterol pool is very dynamic and labile. ER cholesterol accumulation triggers cell death.

W-PO40025

PREVALENCE OF CORONARY CALCIFICATION AND ITS ASSOCIATION WITH INFLAMMATORY MARKERS IN HEMODIALYSIS PATIENTS

DANIELE M. FENSTERSEIFER[1], CRISTINA KAROHL[1], PAULO R SCHVARTZZMAN[2], CESAR AMAURI R COSTA[1], FRANCISCO JOSE VERÍSSIMO VERONESE[1]
Federal University of Rio Grande do Sul and Renal Division, Hospital de Clínicas de Porto Alegre[1], Radiology Service of Hospital Mãe de Deus[2]

Introduction: Patients with end-stage renal disease have a high cardiovascular morbility and mortality. In this cross-sectional study we evaluated the prevalence of coronary calcifications (CC) and its association with clinical parameters and inflammatory markers in hemodialysis patients.
Patients and Methods: Forty-six adult patients in hemodialysis for at least 3 months with cardiac sinus rhythm were included. Patients with acute coronary disease, infections and rheumatic diseases were excluded. Clinical and laboratory data were reviewed, including presence of diabetes, coronary heart disease, body mass index (BMI), calcium (Ca) × phosphorus (P) product and intact parathormone (PTHi) levels. We measured serum lipids, Ca, P, PTHi, albumin (Alb), C-reactive protein (PCR) and fibrinogen (Fb). CC were measured by multislice spiral CT in Agatston (Siemens, Dual Slice), and patients were divided in 2 groups according to the presence (G1: ≥10) or absence (G2: <10) of calcifications.
Results: Calcifications were found in 67% of the patients, with calcification scores (CaSc) showing distribution range = 31.7 (median; P25:0, P75:589.7; minimum: 0; maximum: 5790). Metabolic parameters were (G1 × G2): P = 6.6 ± 1.6 × 6.4 ± 1.6 (P = NS); Ca × P product = 60 ± 20 × 58 ± 15, P = NS; PTHi = 480 ± 4432 × 513 ± 447 (P = NS); PCR = 6.63 (Pe25: 3.90; Pe75: 16.6) × 4.05 (Pe25: 3.2; Pe75: 12.8), P = NS; Fb = 476 ± 77 × 437 ± 106 (P = NS); Alb = 4.27 ± 0.4 × 4.37 ± 0.4 (P = NS). A significant association was found between higher CaCs and age, BMI and PCR levels:

CaSc	Median	25 percentil	75 percentil	P
Age ≥ 50 y	797.0	11.45	1884.2	0.002
<50 y	11.0	0	50.35	
BMI ≥ 25	143.0	2.73	1541.9	0.04
<25	10.0	0	108.35	
PCR ≥ 5	95.0	0	1794.6	0.02
<5	23.0	0	109.7	

Conclusions: Prevalence of CC was high in these patients. Patients over 50 years, obesity and presence of a microinflammatory state were associated with higher CaSc. However, no association was found between CaCs and diabetes, Ca × P product, serum levels of P, PTHi and lipids.

W-PO40026

RISK FACTORS FOR ATHEROSCLEROSIS AMONG CHRONIC HAEMODIALYSIS PATIENTS

CHRISTINA AMIRA[1], S NAICKER[2], K SLIWA-HAHNLE[3], A MIA[3], P MANGA[3]
Department of Medicine, University of Lagos, Nigeria[1], Division of Nephrology, University of Witwaterland[2], Division of Cardiology, University of Witwatersrand[3]

Atherosclerotic cardiovascular disease (CVD) is the major cause of morbidity and mortality in haemodialysis patients.
Aim: To determine the prevalence of traditional and non-traditional risk factors for CVD in our haemodialysis patients and to examine the relationship between these risk factors and the presence of atherosclerosis.
Methods: A cross sectional study of chronic haemodialysis patients: biodata and medical history was obtained via a questionnaire; high sensitivity C-reactive protein (Hs-CRP), homocysteine, adiponectin and lipoprotein(a) were measured. Common carotid intima media thickness (CIMT) was measured by high resolution B-mode ultrasonography as a surrogate marker of atherosclerosis.
Results: 84 patients and 63 age and sex matched controls were recruited, 44 male and 40 female patients and 30 male and 33 female controls. Mean age was 40.27 ?1.13 years for male patients and 40.5 ?11.09 years for male controls; mean age for female patients was 39.83 ?9.69 years and 40.03 ?10.78 years for female controls. Duration on dialysis was 3 mths–16 yrs. Systolic and diastolic blood pressure, Hs-CRP, homocysteine, adiponectin levels were significantly higher among patients compared with controls (p < 0.0001); controls had significantly higher levels of total cholesterol (p < 0.0001), triglycerides (p = 0.04) and LDL cholesterol (p < 0.0001). Patients had higher CIMT than controls (mean of 0.65 ?0.16 mm and 0.61 ?0.15 mm respectively) but this was not statistically significant (p = 0.137). Risk factors associated with CIMT were age (p < 0.001), LDL cholesterol (p = 0.017) and Hs-CRP (p = 0.057). Thirty-two patients (38.1%) had plaques while five controls (7.93%) had plaques. We found no relationship between CIMT and duration on dialysis, calcium, phosphate, parathyroid hormone and albumin levels in the haemodialysis patients.
Conclusion: Some traditional risk factors do not appear to play a role in atherosclerotic CVD in our haemodialysis patients; inflammation and age appear to play an important role.

W-PO40027

INSULIN RESISTANCE, INFLAMMATION AND HYPOADIPONECTINEMIA CORRELATES WITH ENDOTHELIAL DYSFUNCTION IN CAPD PATIENTS

HOON YOUNG CHOI[1], HYUNG JONG KIM[1], TAE HYUN YOO[1], DONG-RYEOL RYU[1], JU SUNG KIM[1], SHIN-WOOK KANG[1], KYU HUN CHOI[1], HO YUNG LEE[1], DAE-SUK HAN[1]
Department of Internal Medicine College of Medicine Yonsei University[1]

ESRD patients undergoing peritoneal dialysis (CAPD) have a high risk of atherosclerotic cardiovascular disease (ACVD) which is not completely explained by conventional risk factors. Insulin resistance (IR) is a well-known feature of PD patients and IR is known to be associated with a premature development of ACVD. Endothelial dysfunction (ED) has been regarded as an early feature of atherosclerosis and plays an important role in the development of atherosclerosis. Recent data reported that endothelial-dependent vasodilation (EDV) is impaired in PD patients. To further ascertain the association between insulin resistance and EDV, we cross-sectionally assessed EDV in 104 non-diabetic CAPD patients and 32 age, sex-matched healthy controls along with determination of IR using the homeostatic model assessment (HOMA-IR) index [plasma glucose (mmol) × (plasma insulin level/22.5)]. Serum adiponectin level and inflammatory markers (hsCRP) were obtained simultaneously. For EDV assessment, flow-mediated dilation (FMD) of the brachial artery was measured using non-invasive Doppler sonography.
Serum insulin and adiponectin level were significantly higher in the PD patients than in the control (9.9 ± 8.8 *vs.* 5.2 ± 3.0 μU/mL, 21.3 ± 8.3 *vs.* 7.9 ± 4.7 μg/mL, $p < 0.05$). HOMA-IR was also significantly higher in the PD group (2.4 ± 2.3 *vs.* 1.1 ± 0.7 [mM·μU/mL], $p < 0.05$). FMD was decreased significantly compared to healthy subjects (10.0 ± 4.8 *vs.* 16.4 ± 4.8%, $p < 0.05$). In PD patients, age, BMI, hsCRP, and HOMA-IR showed a significant negative correlation with FMD (r = –0.232, r = –0.204, r = –0.226, r = –0.231, p < 0.05), whereas serum adiponectin level showed a significant positive correlation with FMD (r = 0.270, p < 0.05). There was a significant positive correlation between hsCRP and HOMA-IR (r = 0.205, p < 0.05). Multiple regression analysis showed that hsCRP concentration was the independent factor affecting FMD in PD patients ($p < 0.05$).
Our observations suggest that insulin resistance, inflammation and hypoadiponectinemia were associated with impaired endothelium-dependent vasodilation. Our data also suggest that inflammation is a potential mediator of IR-associated ED.

W-PO40028

MANAGEMENT OF HYPERTENSION IN PATIENTS ON CHRONIC HAEMODIALYSIS: MODULATION OF DIALYSATE SODIUM COMPARED TO ADJUSTMENT OF DRY WEIGHT AS DETERMINED BY BIOIMPEDANCE ANALYSIS

NAZATUL SHIHA BACHTIAR[1], KHADIJAH KHAIRUDDIN[1], NICHOLAS HOENICH[2], RUBY HUSSAIN[1], SI-YEN TAN[3]
Dept of Physiology, University of Malaya[1], School of Clinical Medical Sciences University of Newcastle UK[2], Dept of Medicine University of Malaya[3]

Hypertension remains a major complication of patients on chronic haemodialysis (HD). HD patients are generally salt overloaded and in many of these patients, the hypertension may be volume dependent. We therefore recently conducted a study to assess the utility of adjusting patients' dry weight as determined by bioimpedance analysis (BIA) of extracellular water/intracellular water (ECW/ICW) ratio compared to adjustment of dialysate sodium and their impact on blood pressure control. Twenty hypertensive HD patients were recruited. The ECW/ICW ratio was measured using Maltron BioScan 916v3 machine and blood pressure, mean arterial pressure (MAP) measured using an automatic Collins blood pressure monitor. Patients were then randomized into 2 groups for intervention. In Group 1, patients' dry weight was adjusted until target ECW/ICW ratio was obtained. In Group 2, dialysate sodium was reduced to 135 mmol/l whilst dry weight was not adjusted unless clinically indicated. All patients were then monitored regularly for changes in blood pressure, MAP and ECW/ICW ratio. Thirty nine percent of randomized patients were female and mean age (±SD) was 49.1 ± 13 years. ECW/ICW ratio was similar for both groups as was MAP. In Group 1, the ECW/ICW was reduced at 2 months (0.8521 vs 0.735, $p = 0.05$). There was also a corresponding fall in blood pressure (103.6 vs 90.0, $p = 0.17$). In Group 2, the reduction in the ECW/ICW (0.96 vs 0.85, $p < 0.05$) was also accompanied by a reduction in MAP (92.7 vs 89.7, $p = 0.355$). The preliminary results of our study suggest that adjusting patients' dry weight to a target predicted by ECW/ICW ratio determined by BIA showed a trend towards improvement in blood pressure control. A similar trend was also observed in patients whose dialysate sodium was reduced. These encouraging preliminary results are being evaluated further with inclusion of larger numbers of patients and with longer duration of follow-up.

W-PO40029

ENDOTHELIAL FUNCTION AND OXIDATIVE STRESS EVALUATION IN HEMODIALYSIS

GANGADHAR TADURI[1], DAKSHINAMURTY KV[1], NAIDU MUR[1], USHA KIRAN[1], KRISHNAMOHAN I[1], PRAVEEN[1]
Nizams Institute of Medical Sciences[1]

Introduction: Dialysis is process where the endothelial function and oxidative stress are dynamically effected.
Aim: To study the effect of hemodialysis on oxidative stress and endothelial function.
Materials and Methods: Prospective analysis.
Dialysis Procedure: Patients who were included in study were having the regular thrice a week hemodialysis. The dialysers and tubing were reused. Reverse osmosis water was used. **Assessment of oxidative stress and endothelial function:** Oxidative stress and endothelial function was assessed before starting, after 2 hours and after 30 minutes of closing the dialysis. Oxidative stress was assessed by using Malonaldehyde (MDA) as the marker. Estimated by using thiobarbituric acid method. Endothelial function was evaluated by using the 'Dicrowin' machine connected to laptop.
Results: Total number of patients included in the study: 12. Males: Females ratio is 8 : 4. Age: 37.5 ± 13.12 years. Height: 164.1667 ± 11.34447 cm; Weight: 56.91667 ± 13.20095 kg. **Oxidative stress:** The basal value of serum MDA is high in dialysis patients in comparison to the healthy control group. (12.3475 ± 3.506019 vs 6.5 ± 1.5). MDA decreased significantly during the dialysis at 21 hrs after hemodialysis (12.3475 ± 3.506019 vs 10.73333 ± 4.331584) and 30 minutes of the post dialysis (12.3475 ± 3.506019 vs 7.479167 ± 2.312781). **Endothelial function:** The basal parameters of endothelial function including reflex index, Stiffness index were high at the start of hemodialysis in comparison to the healthy controls (RI: 60.4 ± 16 vs 58 ± 16; SI: 9.95 ± 2.02 vs 7.5 ± 2).
During the dialysis at 2 hours the RI increased by 5% and the SI increased by 6%. 30 minutes after end of dialysis the RI increased by 10% and the SI increased by 6%.
Conclusion: 1. Dialysis patients have baseline endothelial dysfunction and increased oxidative stress. 2. Oxidative stress decreased significantly during the hemodialysis. 3. Endothelial function did not worsen in a statistically significant manner during the dialysis.

W-PO40030

THE RELATIONSHIP OF SUBCLINICAL HYPOTHYROIDISM AND CARDIOVASCULAR RISK FACTORS IN HEMODIALYSIS PATIENTS

JOON SEUNG LEE[1], MYUNG SOOK CHOI[2], AE KYUNG CHO[2], SEUNG MIN YOO[1], HYNU HEE LEE[1], WOO KYUNG CHUNG[1]
Department of Internal Medicine, Gachon Medical School, Gil Medical Center, Republic of Korea[1]*, Hemodialysis unit, Gachon Medical School, Gil Medical Center*[2]

We investigated the relation between the cardiovascular risk factors and subclinical hypothyroidism (SH) in hemodialysis patients. In this cross sectional study, One hundred fifty patients were examined under hemodialysis over 2-month period. The blood pressure (BP), body mass index (BMI), and fasting thyrotropin (TSH), free thyroxine (fT4), triiodothyronine (T3), total cholesterol (TC), Triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), Apolipoprotein A1 (apoA1), high sensitivity C-reactive protein (hsCRP) were measured. Aortic atherosclerosis was assessed on a lateral X-ray film of the lumbar spine by detecting calcified deposits in the abdominal aorta. SH was defined as an elevated TSH (>4.05 uIU/L) and a normal fT4 and T3 level. Of the 150 patients, 12 (8%) had SH of which 4 were males and 8 were females. Ninety-three patients (63%) were euthyroid (E). The age, sex, BMI, smoking habits, duration of dialysis, and prevalence of diabetes were similar in the SH and E groups. The diastolic BP was significantly higher in SH group compared to the E group ($p = 0.02$). The percentage of the patients with SH having elevated hsCRP (>0.5 mg/dL) and diastolic hypertension (>90 mmHg) was higher than that of the E group ($p = 0.02$, $p = 0.01$ respectively). The only diastolic BP is related with the TSH level ($r = 0.23$, $p = 0.01$). In contrast, there was no significant difference in the TC, TG, HDL-C, LDL-C, apoA1, hsCRP levels between the two groups. The prevalence of aortic calcification between the two groups was not different. In conclusion, the prevalence of SH in the hemodialysis patients is similar to that of the general population, and the SH in the hemodialysis patients appear to increase the risk of atherosclerosis and cardiovascular disease, considering that SH patients had higher diastolic pressure and elevated hsCRP.

W-PO40031

RISK FACTORS FOR HYPERTENSION DEFINED BY AMBULATORY BLOOD PRESSURE MONITORING IN PEDIATRIC PATIENTS ON CHRONIC DIALYSIS

CHAEY-MOON QUEK[1], XIU-MIN LIN[2], CHIEN-WYEI LIEW[1], KEE-SENG CHIA[3], PEI-PEI FOONG[1], YANG-CHERN LIM[4], YEW-WENG LAU[4], HUI-KIM YAP[2]
Shaw-NKF Children's Kidney Centre, The Children's Medical Institute, National University Hospital, Singapore[1]*, Department of Pediatrics, National University of Singapore, Singapore*[2]*, Department of Community Occupational and Family Medicine, National Universtiy of Singapore, Singapore*[3]*, Department of Pediatrics, National University Hospital, Singapore*[4]

Control of hypertension, a major risk factor for cardiovascular morbidity, is important in the management of children with end-stage renal failure. This study aimed at determining the factors that may contribute to hypertension as defined by ambulatory blood pressure (ABP) parameters in pediatric patients on chronic dialysis. Twenty-three patients (mean age 16.4 ± 5.3 years; mean dialysis duration 3.8 ± 2.8 years) were recruited. 24-hour ABP monitoring was performed monthly for 3 months, and systolic (SBP) and diastolic (DBP) parameters studied were mean 24-hour BP, mean wake and sleep BP, 24-hour BP loads, wake and sleep BP loads, and nocturnal systolic and diastolic dipping. These parameters were subsequently correlated with body mass index, dialysis duration, mean clinic BP measurements, mean monthly levels of calcium, phosphate, intact PTH, calcium-phosphate product, hematocrit and uric acid. Multivariate linear regression analysis was performed using the ABP parameters as dependent variables. Mean clinic SBP was shown to correlate significantly with all the ABP parameters except for sleep DBP, and nocturnal SBP and DBP dips. Mean clinic DBP was significantly correlated with all the ABP parameters of DBP except DBP dip. Linear regression analysis showed that mean hematocrit, which reflects volume status in dialysis patients, did not correlate with clinic BP, but correlated strongly inversely with several ABP parameters including 24-hour mean SBP, sleep SBP and DBP, 24-hour SBP and DBP loads, and wake SBP and DPB loads ($p < 0.05$). The mean calcium-phosphate product correlated inversely only with the nocturnal SBP dip ($p = 0.003$), suggesting that vascular calcifications could be the cause of early hypertension as defined by the loss of nocturnal dip. In conclusion, control of hypertension as measured by ABP parameters may be more important in managing pediatric patients on chronic dialysis, as these reflect the contributory pathophysiological factors more accurately than clinic BP.

W-PO40032

A PROSPECTIVE, RANDOMIZED, PLACEBO-CONTROLLED STUDY TO EVALUATE THE EFFECT OF COMBINED TREATMENT OF FOLIC ACID AND INTRAVENOUS B-COMPLEX VITAMINS ON HYPERHOMOCYSTEINEMIA IN HEMODIALYSIS PATIENTS

LI PENG[1], QIAN LIU[2]

Department of Nephrology, The 5th Affiliated Hospital of Sun Yat-Sen University, China[1], The 5th Affiliated Hospital of Sun Yat-Sen University[2]

Background: Hyperhomocyteinemia is an important risk factor for cardiovascular morbidity and mortality in hemodialysis (HD) patients. Some studies showed that folic acid (FA) supplement significantly reduced but not normalized the plasma homocysteine (tHcy) in HD patients. Several studies testing the ability of VitB12 or B6 in lowering tHcy in HD patients produced conflicting results. Whether combined treatment of FA with intravenous B-complex vitamins (BCV) will more effectively lower the plasma tHcy is not yet well established. Objective: To evaluate the effect of combined treatment of FA with BCV on lowering plasma tHcy in HD patients.

Method: 48 patients who had stably undergone HD > 3 months were enrolled. After washout from previous FA or VitB therapy, all patients were given FA 5 mg/day orally for 3 months. Then patients were randomly allocated into two treatment arms(n = 24), FA 5 mg/day with either intravenous BVC (B1 250 mg + B6 250 mg + B12 1.5 mg) or intravenous saline thrice a week for 3 months. Pre-dialysis plasma tHcy, FA, VitB12 levels, hemoglobin and serum albumin were determined at the end of washout, run-in and treatment period respectively.

Results: The mean value of tHcy decreased from 28.5 ± 11.7 μmol/L to 19.6 ± 5.8 μmol/L (31%, P < 0.001) after 3 months of FA treatment. Combined treatment of FA with BCV had an additional decrease in tHcy by 13.6% as compared with control group (4.9%) (15.8 ± 6.1 μmol/L vs 18.2 ± 7.4 μmol/L, P < 0.05). Ten patients (41.7%) in combined treatment group achieved normal tHcy and only one patient (4.2%) in the control group achieved normalized tHcy (P < 0.001).

Conclusion: Mild to moderate hypercysteinemia was present in all of the studied patients. FA has been confirmed to be an effective treatment for hypercysteinemia. Combined treatment of FA with intravenous BCV could be more effectively to achieve normal homocysteine in HD patients.

W-PO40033

SERUM OSTEOPROTEGERIN LEVELS AND ABDOMINAL AORTIC CALCIFIC DEPOSITS IN MAINTENANCE HEMODIALYSIS PATIENTS

HYANG KIM[1], HYUN YOUNG LHEE[1], KYU-BECK LEE[1], YOUNG RAE LEE[2]

Department of Internal Medicine, Sungkyunkwan University School of Medicine, Kangbuk Samsung Hospital. Korea[1], Department of Radiology, Sungkyunkwan University School of Medicine, Kangbuk Samsung Hospital, Korea[2]

Vascular calcification is highly correlated with cardiovascular disease mortality, especially in patients with end-stage renal disease (ESRD). Recently, evidence is accumulating that osteoprotegerin (OPG) may involve vascular calcification. The present study aims to examine whether serum OPG is associated with AAC deposits by lateral lumbar radiograms in maintenance hemodialysis patients.

We studied 57 ESRD patients on maintenance hemodialysis. Serum OPG level was measured by enzyme-linked immunosorbent assay. All patients underwent lateral lumbar radiograms. Anterior and posterior wall calcific deposits in the aorta at the level of the first through fourth lumbar vertebrae were graded individually on a 0–3 scale according to increasing severity using a previously validated rating scale for AAC in a Framingham Heart Study subpopulation. The values were summed, resulting in an AAC index that could ranges from 0 to 24 points. Patients were classified into tertile groups according to their AAC index: group I (0, n = 18), group II (1–4, n = 18) and group III (5–18, n = 21).

The results are as follows: 1) Serum OPG level was higher in patients with higher AAC index than in those with lower AAC index (group I: 409.8 ± 343.5 pg/ml, group II: 575.9 ± 723.3 pg/ml and group III: 660.8 ± 593.5 pg/ml), but it was not statistically significant ($p = 0.365$). 2) Patients with higher AAC index showed higher frequency of past history of cardiovascular disease (group I: 5.6%, group II: 22.2% and group III: 33.3%, $p = 0.036$) and hyperlipidemia (group I: 5.6%, group II: 5.6% and group III: 33.3%, $p = 0.016$). 3) Serum OPG levels had positive correlation with age (r = 0.336, $p = 0.011$) and pulse pressure (r = 0.291, $p = 0.028$). 4) AAC index showed positive correlation with age (r = 0.380, $p = 0.004$) and WBC counts (r = 0.278, $p = 0.036$).

In conclusion, we suggest that serum OPG might be helpful clinical marker for the development of vascular calcification in maintenance hemodialysis patients.

W-PO40034

SEASONAL VARIABILITY IN BLOOD PRESSURE IN HEMODIALYSIS PATIENTS IN ARGENTINA

ADRIAN M. GUINSBURG[1], FELIPE INSERRA[1], CRISTINA MARELLI[1], JUTTA PASSLICK-DEETJEN[2]

Fresenius Medical Care, Argentina[1], Fresenius Medical Care, Germany[2]

Background: Seasonal variability in blood pressure has been reported in the general population and hypertensive patients but in patients undergoing hemodialysis it is uncertain. The aim of this study was to analyze seasonal variations in blood pressure in hemodialysis patients treated at Fresenius Medical Care dialysis units in Argentina.

Methods: We analyzed retrospectively 188,951 blood pressure measures from 7,793 patients undergoing hemodialysis in 97 dialysis centers in Argentina during 45 months. We collected predialysis systolic blood pressure (SBP), predialysis diastolic blood pressure (DBP), interdialytic weight gain (IDWG) and mean arterial pressure (MAP). Warmer months (January, February and March) were compared to colder months (June, July and August) using paired samples t-test. Linear regression was performed to analyze the association between blood pressure levels and mean ambient temperature (MAT) with adjustment for age, gender, diabetic status and IDWG.

Results: SBP, DBP, MAP and IDWG were higher in winter (table).

	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)	IDWG (kg)
Winter	134.9 ± 24	75.6 ± 14	95.3 ± 16	2.42 ± 1.1
Summer	132.3 ± 24	74.4 ± 14	93.7 ± 16	2.31 ± 1.1
D Mean	2.6	1.2	1.6	0.11
p	<0.0001	<0.0001	<0.0001	<0.0001

Multivariate regression analysis showed inverse correlation between SBP and MAT (beta −0.42 $p < 0.0001$) after controlling for age, gender, diabetic status and IDWG.

Conclusions: Blood pressure in hemodialysis patients in Argentina varies seasonally with higher values in the winter and lower values in the summer. Blood pressure levels are inversely related to mean ambient temperature and this effect is independent of IDWG levels.

W-PO40035

ADMA DECREASES CEREBRAL BLOOD FLOW IN HEALTHY HUMANS: A RANDOMISED CONTROLLED STUDY USING DYNAMIC CONTRAST-ENHANCED PERFUSION MRI OF THE BRAIN

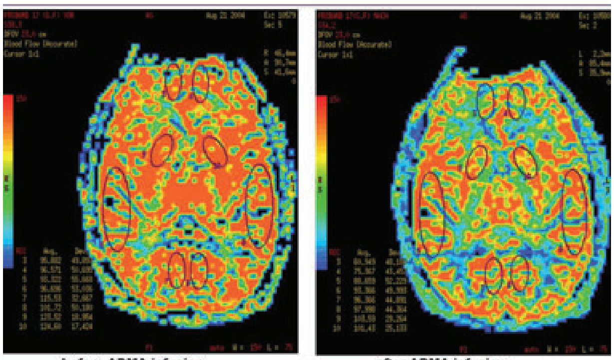
JAN T. KIELSTEIN[1], FRANK DONNERSTAG[2], SANDRA GASPER[1], HERMANN HALLER[1], DANILO FLISER[1], STENFANIE M. BODE-BOEGER[3]

Department of Nephrology, Medical School Hannover, Germany[1], Department of Neuroradiology, Medical School Hannover, Germany[2], Institute of Clinical Pharmacology, University Hospital, Otto-von-Guericke -University Magdeburg, Germany[3]

Background: Several in vitro studies as well as animal studies suggest that the endogenous NO-synthase inhibitor asymmetric dimethylarginine (ADMA) can increase vascular tone in cerebral blood vessels. Marked elevation ADMA had been shown in diseases with decreased cerebral perfusion i.e. ischemic stroke and Alzheimer's disease. Purpose of this study was to investigate the influence of systemic ADMA infusion on cerebral perfusion in humans and on arterial stiffness, increasingly recognized as an important cardiovascular risk factor.

Patients and Methods: Twenty healthy Caucasian men were randomized to infusion of either placebo or ADMA (0.10 mg $ADMA \cdot kg^{-1} \cdot min^{-1}$). Volunteers underwent measurement of cerebral perfusion using dynamic contrast-enhanced perfusion MRI of the brain. Before and 20 min after the infusion period (40 min) MRI was performed using a GE Signa Horizon 1.5 Tesla tomograph. Cerebral blood flow, visualized by the dynamic imaging of an intravenously injected bolus of gadolinium-containing contrast media, was measured in several regions of interest (ROIs). Augmentation index (augmented pressure/pulse pressure) was measured by applanation tonometry (Sphygmocor).

Results: Infusion of ADMA (0.10 mg ADMA·kg^{-1}·min^{-}) over a period of 40 min significantly decreased cerebral perfusion by 15.1 ± 4.5% ($p = 0.014$) whereas the blood flow in the placebo group tended to increase by 7.7 ± 2.8%. Furthermore Typical MRI pictures before (left panel) and after (right panel) the infusion of ADMA with a decrease in blood flow by 15% is shown in the Figure. Infusion of ADMA significantly ($p = 0.0068$) increased the augmentation index.
Conclusions: ADMA may be an important endogenous modulator of cerebral vascular tone under resting conditions. Increased levels of ADMA, as frequently observed in patients with ESRD, may be involved in the pathogenesis of ischemic stroke and cognitive impairment in patients with chronic renal disease.



before ADMA infusion after ADMA infusion

W-PO40036

A FUNCTIONAL POLYMORPHISM IN THE PROMOTER REGION OF THE GENE ENCODING FOR INTERLEUKIN-6 (IL-6) IS ASSOCIATED WITH INCREASED PLASMA LEVELS OF HIGH DENSITY LIPOPROTEIN (HDL) IN PATIENTS WITH END-STAGE RENAL DISEASE (ESRD)

JONAS AXELSSON[1], OLOF HEIMBÜRGER[1], PETER BÁRÁNY[1], BENGT LINDHOLM[1], LOUISE NORDFROS[2], PETER STENVINKEL[1]
Department of Renal Medicine and Baxter Novum, Karolinska Institute, Sweden[1], Center for Molecular Medicine, Karolinska Institute, Sweden[2]

Background: LCAT is a key enzyme of reverse cholesterol transport and Feister et al. have demonstrated that an IL-6 responsive transcription factor contributes to LCAT transcription regulation. We have recently shown that IL-6 SNPs influence inflammation in patients with ESRD, and in the present study we investigate the potential impact of this on serum HDL.
Patients and Methods: In a cohort of 204 (62% males) ESRD patients aged 52 ± 1 years and with a mean GFR of 7.2 ± 0.2 ml/min the following parameters were studied: age, presence of CVD, serum IL-6, triglycerides, LDL-cholesterol, HDL-cholesterol, glomerular filtration rate (GFR), lipid modifying medication and single nucleotide polymorphisms (SNPs) in the IL-6 gene at positions −572 and −174 using Pyrosequencing® (Pyrosequencing AB, Stockholm, Sweden).
Results: Serum IL-6 was correlated with HDL (rho = −0.16; $p < 0.01$). In the three IL-6 −174 genotype groups, we found no significant differences in patient age, GFR or in levels of TG, LDL, prevalance of CVD or in the use of lipid lowering medications. Plasma levels of HDL where significantly higher in the IL-6 −174 C/C genotype group compared to the G/C and G/G groups (1.34 vs 1.17 and 1.13 mmol/L; $p < 0.05$) while the differences in IL-6 where non-significant. In inflamed (n = 68), but not in non-inflamed, patients serum levels of both IL-6 (14.3 ± 0.2 vs. 10.2 ± 0.1 and 12.0 ± 0.1 ng/mL respectively; $p < 0.05$) and HDL (1.3 ± 0.1 vs. 1.0 ± 0.1 and 0.8 ± 0.1 mmol/L respectively; $p < 0.01$) where elevated in the −174 C/C genotype group compared to the G/C and G/G groups. We found no significant differences in any of the studied parameters between the IL-6 −572 SNP genotype groups.
Conclusion: We conclude that the genotype at position −174 in the IL-6 gene may play a role in influencing plasma HDL levels in patients with ESRD.

W-PO40037

ENDOTHELIAL DYSFUNCTION AND MORTALITY IN PATIENTS WITH HEMODIALYSIS

GUILLERMO IBAÑEZ[1,2], ROSA DIEZ G.[2], PIZARRO R.[2], OBERTY P.[2], FALCONI M.[2], DEL PINO N.[3], GUINSBURG A.[4], ALGRANATI L[2]
Hemodialysis, Fresenius Medical Care Sgo Del Estero, Argentina[1], Nephrology and Cardiology. Italiano Hospital[2], BIOSIDUS Laboratory[3], Fresenius Medical Care[4]

Introduction: The first cause of death in patients undergoing hemodialysis (HD) is of cardiovascular origin. Atherosclerosis is an accelerated process for people undergoing (HD) and the first symptom is endothelial dysfunction (DE).
Objectives: To determine whether there is a link between (DE) and mortality in (HD).
Methods and Patients: We studied prospectively 86 patients (2002–2004). The mean age was 59/16 (59% male) having undergone hemodialysis for an average of 51 months. 32 patients died (37.2%); 7 were transplanted (8.14%). The (DE) values had been measured at the start, considering DE as the difference between the humeral artery diameter <15% before and after the stimulus. Those values were inferred through ultrasonography (doppler) humeral and carotid, measuring (VRE) induced by ischemia, and (IMC). Humoral dosage was performed of 16 main substances, and 10 variable clinical factors were included. The final point considered was mortality. $p < 0.005$ was considered as significant.
Results: Correlation between (IMC) and (DE) and the humoral variables was weak. Only correlation between (IMC) and (DE) and age (>70 years old) was significant. In the monovaried analysis (DE) was linked to mortality significantly. The main predictors were: males, age >70 years old, and the albumin <4 g/dl.
Conclusion: (DE) is not linked to mortality independently, and neither are the humoral variables. Only the clinical variables and the plasmatic albumin concentration were important prognosis predictors. The plasmatic hyperhomacysteinemia was not correlated with mortality either – This data is useful because it allows us to stratify the risk with patients.

W-PO40038

METABOLIC SYNDROME IN HEMODIALYSIS PATIENTS

LYGERI PAULOS SOUBASSI[1], EMMANUEL PAPADAKIS[1], ILIAS THEODOROPOULOS[1], THEODOROS CHIRAS[1], MARIA DOULI[1], IOANNIS TSAPAKIDIS[1], STYLIANOS N ZEREFOS[1], GRIGORIOS DAGLAS[1], CHRISTOS KALIANOS[1], DIMITRIS VALIS[1], NIKOLAS ZEREFOS[1]
Renal Unit, Hygeia Hospital, Greece[1]

The metabolic syndrome (MS) is associated with increased risk for cardiovascular disease (CVD).
Aim: To define the frequency of MS among our hemodialysis (HD) patients (pts) and evaluate the efficiency of management according to current guidelines.
Methods: One hundred and eighty five (M = 114, F = 71) HD pts, aged 66.6 ± 12.7, have been on chronic HD in our unit for 50.4 ± 43.2 mo.
They were evaluated for the following factors: BMI, waist circumference, TG, HDL, fasting blood glucose (FBG), detailed medical history, LDL levels, smoking, hypertension, coronary artery disease (CAD), diabetes mellitus (DM), hyperlipidemia and according to the ATPIII criteria for the diagnosis of MS.
Results: Of all our pts: 19% were smokers, 33% were hypertensive, 32% had documented CAD, 25% had DM, 25% were hyperlipidemic. 41.6% were overweight or obese (BMI > 30), mostly women 20.8% vs 10% of men, 7.6% had LDL > 130, mostly men (2.2% of vs. 1.1 of women, with LDL = 173.5 ± 41.6 vs. 138.2 ± 2.5 respectively, 6% were on lipid lowering therapy (LDL = 104 ± 56). 6.5% were not aware about their abnormal FBG levels, 33% had BP ≥ 130/^{3}85 mmHg and were receiving antihypertensive treatment.
Forty six (24.9%) had MS (Group A), 74% men, 26% women, while 139 (75.1%) (Group B) did not present the above syndrome.
The results are summarized in the following table:

Group		BMI		Waist circumference		TG		HDL		FBG	
		Value	>30	Value	>102(M), >88(F)	Value	≥150	Value	<40(M), <50(F)	Value	≥110
A	M	33.5 ± 2.4	4.9%	113 ± 8.5	19%	262 ± 90	12.4%	30.8 ± 3.7	11.9%	169 ± 53	9%
	F	34.2 ± 2.9	3.2%	99.6 ± 9.9	5.9%	271 ± 137	3.8%	34.5 ± 4.2	4.3%	165 ± 62	2%
B	M	32.8 ± 2.5	6%	110.8 ± 7.7	18.3%	261 ± 102	27%	31.8 ± 4.5	23.2%	161 ± 46	22%
	F	33 ± 1	1.6%	98.9 ± 8.3	15.13%	235 ± 99	13.5%	43 ± 5	3.8%	156 ± 54	8.6%

Statistically significant differences between Group A and B were found for: waist circumference in men, TG, HDL, FBG.
Conclusion: We believe that the successful treatment of these metabolic alterations may improve the survival of HD pts.

W-PO40039

BENEFICIAL EFFECTS OF SEVELAMER ON CARDIOVASCULAR RISK FACTORS IN HEMODIALYSIS PATIENTS

LYGERI PAULOS SOUBASSI[1], EMMANUEL PAPADAKIS[1], ILIAS THEODOROPOULOS[1], MARIA DOULI[1], STYLIANOS NIKOLAOS ZEREFOS[1], IOANNIS TSAPAKIDIS[1], HEODOROS CHIRAS[1], T ALEKSANDROS KOUVELIS[1], GEORGE POULOS[1], GRIGORIOS DAGLAS[1], CHRISTOS KALIANOS[1], SOFIA SOUBASSI[1], DIMITRIOS VALIS[1], NIKOLAOS STYLIANOS ZEREFOS[1]
Renal Unit, Hygeia Hospital, Greece[1]

Cardiovascular disease is frequent and severe in patients (pts) on hemodialysis (HD) and has recently been attributed to vascular calcification.
Aim: To determine the effect of sevelamer in chronic hemodialysis (CHD) pts with different types of membranes (M) on the lipid profile, chronic inflammation and adequacy of HD.
Methods: The study included 102 pts (62 M and 40 F) aged 66 ± 13 yo on regular CHD for 50.4 ± 44.4 mo. Two different types of M were randomly used: a) cellulose based (CBM) in 47 pts and b) synthetic polysulfone (SPM) in 55 pts, continuously for 3 consecutive mo. The pts were divided in two groups: Group A 38 pts taking only sevelamer and Group B on standard calcium based phosphate binders.
According to the type of M used Group A pts were subdivided in subgroup A1 on CBM (18 pts) and subgroup A2 on SPM (20 pts). Group B pts were also subdivided in subgroup B1 on CBM (29 pts) and subgroup B2 on SPM (35 pts). The **results** are summarized in the following table:

HD	Sevelamer (Group A)		Calcium (Group B)	
	A_1 CBM n = 18	A_2 SPM n = 20	B_1 CBM n = 29	B_2 SPM n = 35
TC (mg/dl)	169.7 ± 35.2	166.6 ± 47.3	179.3 ± 38.2	167 ± 44
TG (mg/dl)	174 ± 99	143 ± 70	196 ± 141	164.3 ± 86
HDL-C (mg/dl)	50.3 ± 16.6	45.3 ± 12	45.6 ± 11.7	45 ± 13.6
LDL-C (mg/dl)	93 ± 41	74 ± 46	93 ± 37.6	90 ± 40
Ca (mg/dl)	9.4 ± 1.1	9.5 ± 1	9.4 ± 0.7	9.1 ± 1.2
P (mg/dl)	4.7 ± 1	5.1 ± 1.6	5 ± 1.5	5.3 ± 1.7
CaxP (mg^2/dl^2)	44.8 ± 20	34.3 ± 28	47.5 ± 17	46.9 ± 17
iPTH (pg/ml)	82.6 ± 55	81.6 ± 77	84.2 ± 56	139 ± 105
CRP hs (mg/dl)	0.48 ± 0.38	0.66 ± 0.5	1.28 ± 1	1.32 ± 0.9
Homocysteine (μmol/L)	22.2 ± 12.3	15.15 ± 12.8	22.3 ± 15	23.5 ± 17.7
Kt/V	1.3 ± 0.3	1.2 ± 0.2	1.23 ± 0.6	1 ± 0.5

HDL is significantly higher in pts dialyzed with CBM only on sevelamer (p 0.05). Serum (s) P was significantly lower in pts dialyzed with SPM on sevelamer (p 0.01). s.CRP hs values were significantly lower in pts dialyzed with SPM on sevelamer (p 0.003). Kt/v was significantly improved in pts dialyzed with CBM on sevelamer (p 0.01). There was no correlation between the two phosphate binders and age, sex, BMI, smoking, coronary artery disease, hypertension and alfacalcidol.
Conclusion: Sevelamer, has effectively lowered s. P levels in HD pts dialyzed with both types of M, but more impressively with SPM. Sevelamer effectively increased HDL levels, has reduced CRP values and increased Kt/v, when CBM were used. The results suggest that sevelamer may have a beneficial effect on cardiovascular risk factors.

W-PO40040

POSSIBLE NEW INDICATION OF LDL-APHERESIS FOR AN ATTENUATION OF ATHEROSCLEROSIS IN PATIENTS WITH ESRD – ITS EVIDENCE FOR REDUCING PRO-INFLAMMATORY BIOMARKERS

SHUZO-KOBAYASHI[1], MACHIKO OKA, KOUJI OKAMOTO, KYOUKO MAESATO, HIDEKAZU MORIYA, TAKAYASU OHTAKE[1]
Shonan Kamakura General Hospital[1]

Introduction: Atherosclerotic cardiovascular disorder has much impact on mortality in patients with ESRD. Atherosclerosis is known to be an inflammation involving in leukocytes, platelets and endothelium. Particularly, P-selectin plays an important role by being an adhesion receptor for leukocyte expressed by activated platelets and endothelial cells. LDL-apheresis (LA) is well known to improve peripheral arterial occlusive disease through an up-regulation of vascular endothelial growth factor (VEGF) (J. Clin, Apheresis 18:115,2003). Moreover, LA is known to show angiographic arrest of the progression of coronary artery disease (Circulation 93:1826,1996). In the present study, we sought to reveal whether or not LA reduces pro-inflammatory biomarkers related with atherosclerosis including P-selectin.
Methods: Sixteen ESRD patients with PAOD were treated with LA (Kaneka, Japan) for 5 weeks (10 sessions). Before and after single session of LA, and prior to LA after 10-times treatment, serum or plasma levels of high sensitive CRP (hsCRP), MCP-1, P-selectin, fibrinogen were measured.
Results: All biomarkers were significantly reduced after a single session of LA, comparing with those before treatment. After 10-times therapy, log (hsCRP) were significantly decreased from 3.66 ± 0.126 to 3.19 ± 0.188. Serum levels of P-selectin was also significantly reduced from 358 ± 202 to 283 ± 183 ng/ml. Plasma fibrinogen levels significantly decreased from193 ± 40 to 152 ± 32 mg/dl, while serum MCP-1 was not changed.
Conclusion: LA significantly reduced serum levels of hsCRP, P-selectin and plasma level of fibrinogen in patients with PAOD after 10-times treatment. LA may have a possibility to attenuate a progression of atherosclerosis in patients with HD.

W-PO40041

INADEQUATE TREATMENT OF CARDIAC DISEASE IN DIALYSIS PATIENTS

ANIL RANCHORD[1], G PIDGEON[1]
Department or Renal Medicine, Wellington Hospital, Wellington, New Zealand[1]

Introduction: Dialysis patients with ischaemic heart disease (IHD) or congestive heart failure (CHF) have a high mortality rate. The aim of this audit was to assess the adequacy of drug treatment for the secondary prevention of IHD and CHF in dialysis patients.
Methods: Dialysis patients served by a single tertiary hospital were included if they had been on stable dialysis for a least 3 months at 1 July 2004. Patients in the IHD arm met criteria for coronary artery disease, myocardial infarction (MI) or coronary intervention within the last 5 years. Patients in the CHF arm had moderate or severe left ventricular systolic dysfunction on cardiac imaging. The drug treatments considered were: aspirin for IHD; beta-blockers post MI, for a positive cardiac stress test or for persistent angina; statins post MI, coronary artery bypass grafting or percutaneous coronary intervention; renin angiotensin aldosterone system blockers (RAAS) and beta-blockers for CHF. Patients who had a documented contraindication to a medication were not included in the analysis for that drug, thereby avoiding the underestimation of treatment use.
Results: Of 211 patients on dialysis (89 haemodialysis, 122 peritoneal dialysis), 50 met the criteria for IHD and 24 for CHF.

Ischaemic Heart Disease

Treatment	Patients treated (%)
Aspirin	86
Beta-blocker	64
Statin	70

Of the patients with IHD who were on treatment for hypertension 29% were not on a beta-blocker.

Heart Failure

Treatment	Patients treated (%)
Beta-blocker	43
RAAS blocker	55
Beta-blocker & RAAS blocker	24
No beta-blocker or RAAS blocker	21

Conclusions: Within this single renal department there was inadequate drug treatment for the secondary prevention of IHD and severe CHF in stable dialysis patients.

W-PO40042

EFFECT OF ACUTE FLUID RESTRICTION ON NATRIURETIC PEPTIDE LEVELS IN PATIENTS WITH HYPERTENSION IN CHRONIC DIALYSIS PROGRAM

IVO ORAL[1], ZBYNĚK ŠIŠLÁK[1], DAVID STEJSKAL[2], JAROSLAV LOUCKÝ[3], ZDENĚK COUFAL[1], JERGUŠ MISTRÍK[1], ROBERT NÁPLAVA[1]

Internal clinic, Bata hospital, Zlín, Czech republic[1], Department of clinical biochemistry, Hospital Sternberk[2], IMALAB, Zlín[3]

30 hypertensive patients in chronic dialysis program with left ventricular hypertrophy, were tested for BNP, NT-proBNP, echocardiography (2D,M-mode, doppler flow), basic biochemistry immediately prior to and following dialysis. Changes in natriuretic peptides as well as in all above mentioned parameters were assessed in relation to the volume of dialysed fluids (3750 ml on average).

Results:

	LKd	EF	LS	E/A	DT	TO	TF	CO	BNP	proBNP
Before	53	0.66	46.29	1.326	201.04	79.88	76.5	6.12	2.85	193.2
After	50.2	0.65	**41.91×**	**0961×**	**226.7×**	**72.1×**	80.1	**5.64×**	**2.61×**	213.7
Calculated values									**3.3×**	226.5

With regard to the thickening of circulating the anticipated concentration of BNP and NT-proBNP in thickened blood was calculated (**BNP 3.30 pg/ml** and **NT-proBNP 226.5 pg/ml**). The decrease of circulating BNP as opposed to the calculated value was, statistically significant (×), while changes in NT-proBNP leves did not reach statistical significance.

Conclusions: 1) Patients presented with increased NT-proBNP levels, but did not show pathological values of BNP at the study entry. 2) The circulating NT-proBNP levels are stable and represent a long-term marker of the interplay between the volume of circulating fluids and the left ventricular function. 3) BNP levels respond fast to changes in the volume of circulating fluids. The changes correlate with changes in left ventricle filling.

W-PO40043

THE PROGRESSION OF CORONARY ARTERY CALCIFICATION (CAC) MEASURED BY ELECTRON-BEAM COMPUTED TOMOGRAPHY (EBCT) IN HEMODIALYSIS PATIENTS

WEIJING BIAN[1], LING ZHANG[1], YIPU CHEN[1], BIN LIU[1]

Center of Nephrology, China-Japan Friendship Hospital, Beijing, 100029, P.R China[1]

Objective: The aim of this study was to investigate risk factors related to CAC and its progression in hemodialysis patients.

Methods: Quantification of CAC was measured by EBCT, known as the CAC score (CACS). We examined the relation between coronary risk factors, metabolism of calcium and phosphorus, and CAC progression.

Results: We studied 22 patients undergoing maintenance hemodialysis. The average age of the patients was 61.18 ± 12.50 year old and duration of hemodialysis was 91.81 ± 62.80 months. The 21 patients (95.4%) had CAC. The mean CACS value was 1935.54 ± 2614.67. Patients with CACS >100 had significantly higher morbidity of cardiovascular diseases, serum phosphate, calcium-phosphate product, C-reactive protein, and lower serum albumin than those patients with CACS <100. In 8 of 22 patients, CACS was measured twice, during a mean interscaning period of 18.87 ± 2.58 months. The mean CACS progressed from 1935.54 to 2672.42, and the mean change in CACS was 420.00 ± 389.73. Multiple regression analysis demonstrated that CAC was associated with duration of hemodialysis and high-density lipoprotein cholesterol, and progression of CAC was associated with high-density lipoprotein cholesterol only.

Conclusions: Our present study suggests that serum phosphate, calcium-phosphate products correlate with CAC. The duration of hemodialysis and high-density lipoprotein cholesterol are independent predictors of CAC, and high-density lipoprotein cholesterol is an independent predictor of CAC progression.

W-PO40044

PREVENTION OF ATHEROSCLEROSIS PROGRESSION BY ANGIOTENSIN RECEPTOR BLOCKER FOR HEMODIALYSIS PATIENTS

MAKOTO KITAMURA[1], SASAO GEN[1]

Tokai University Hospital[1]

Angiotensin receptor blocker (ARB) is known by the effects to prevent atherosclerosis progression in hypertension patients. We investigated the effects of ARB in maintenance hemodialysis patients.

Patients and Methods: Daily 40–80 mg of valsartan has been given to six patients undergoing hemodialysis including three diabetes patients for one year (ARB group). Thirteen patients undergoing dialysis including five diabetes in a same clinic have not given ARB (Control group). Pulse wave velocity (PWV) was monitored both groups before and after the observation period. PWV was monitored by ABI form (Colin Co. Ltd.). Drugs for anti-hypertension and anti-hypercholesterolemia were not changed in all patients for the observation period.

Results: In ARB group, two of six patients showed decrease of PWV and no patients increase PWV. In control group, four of 13 patients showed increase of PWV and one patients decrease PWV.

Conclusion: ARB showed prevention effects to atheroscrelosis progression in hemodialysis patients.

W-PO40045

THE IMPORTANCE OF T TROPONINE TEST IN MYOCARDIAL DISORDERS QUANTIFICATION TO ERSD PATIENTS

AURELIAN-OVIDIU SIMIONESCU[1], LIVIA SIMIONESCU[1], G. BOERU[1], GEANINA BOERU[1]

County Hospital, Slatina, Romania[1]

We took in our research (1st october 2003–31 dec 2004) 42ERSD patients in hemodialysis program and 20 CRF patients with creatinine more than 5 mg/dl. The T troponine test was gathered at 3, 6, 12 months.

We noted the follows major cardiac (MCE) events at ERSD: ventricular rhythm disorders with malign potential, the emphasize of NYHA class cardiac failure, cardiac pulmonary edema, acute coronarian syndrome.

Results:

	Months of hemodyalisis	Troponine ng/dl
ESRD	3 months	0.35 ± 0.3
	6 months	0.49 ± 0.4
	12 months	0.67 ± 0.4
average		0.47 ± 2.3
CRF	—	0.21 ± 0.03

Months of hemodyalisis	Total nr of patients	Patients with MCE	%	Troponine ng/dl
3	42	3	7.14	1.55 ± 0.3
6	42	8	19.05	1.68 ± 0.2
12	42	15	35.71	1.67 ± 0.4
Average				1.62 ± 0.4

Conclusions: The hemodialysis is a suplementary factor of deterioration on the miocard function and structure, showed by the increase of the T troponine comparing with CRF patients.

The jump of T troponine 3–4 times more than basis level is a bad prognosis, showing the unfurling of the cardiac events with bad prognosis including death. Is necessary to take some immediately and intensive therapeutical measures, to decrease the importance of cardiac events.

The observation of T troponine to the ERSD patients is necessary for surprising the T troponine jump level, which express the acute deterioration of the miocard muscle and it can appreciate the cardiac risc better than the biological methods. EKG, Ecocardiograme.

The T troponine test is easy to do, the result is quickly (15 minutes) and the informations correctly interpreted may limit the segment of patients with a high cardiac risc who are needing immediately intensive treatement measures.

W-PO40046

PERIPHERAL ARTERIAL OCCLUSIVE DISEASE, CLINICAL MANIFESTATION AND RISK FACTORS AMONG PATIENTS WITH REGULAR HEMODIALYSIS

KETUT SUWITRA[1], RAKA WIDIANA[1], IG WULANDARI[1], LOEKMAN JS[1], SUDHANA IW[1]
Department of Internal Medicine Faculty of Medicine Udayana University Bali Indonesia[1]

Background: Peripheral Arterial Occlusive Disease (PAOD) is common among patients undergoing hemodialysis (HD). In the general population, DM, male gender, cigarette smoking, advanced age, hypertension and dyslipidemia are the most important risk factors for PAOD. Some factors such as hyperhomocysteinemia and Ca-P product are also considered as risk factors
Study Objective: To evaluate prevalence of PAOD and association with risk factors among patients who are undergoing regular HD.
Method: A cross-sectional study was done on 46 regular HD patients. Morning fasting blood samples were taken for lipid profile, blood sugar, blood urea nitrogen, serum creatinine, albumin levels, Homocysteine (Hcy) levels, Ca, inorganic P. Ankle-brachial index (ABI) of blood pressure was measured after dialysis session using Va Sera VS-100.
Result: Fourty-six (26 males, 20 females) regular HD patients, aged 50.95 ± 9.90 years, duration of HD 27.7 ± 22 months were included in this study. Using ABI <0.9 as cut off value for the presence of PAOD, 10/46 (22%) of HD patients had PAOD. With regard to risk factors, DM were found in 9/46 (20%); smoking in 7/46 (15%); dyslipidemia in 41/46 (89%); hypertension in 20/46 (43%); obesity in 5/46 (11%), age >50 years 22/46 (48%; CaXP >55 in 21/46 (46%); Hyper-Hcy in 30/46 (65%). With regard to clinical manifestation of PAOD, intermitten claudication were found in 14/46 (30%); pain at rest in 6/46 (13%); leg ulcer in 5/46 (11%), and gangrene in 2/46 (4%). Using logistic regression analysis to analyze 8 risk factors, some risk factors for PAOD which considered to havie clinical importance were hyper-Hcy (OR 3.46), normotension (OR 0.35), non DM (OR 0.26), male gender (OR 3.09), and smoking habit (OR 2.00).
Conclusion: More than twenty percent of patients with regular HD had POAD. Hyper-Hcy male gender and smoking habits are risk factors for PAOD whereas normal bloon pressure and non-DM are protective factors.

W-PO40047

HEMODIALYSIS PATIENTS: FETUIN-A AND OTHER RISK FACTORS OF CARDIAC CALCIFICATION

MICAELA MANNI[1], A. BALDUCCI[1], L. JANKOVIC[2], M. MOROSETTI[2], D. MANTELLA[3], A. NATICCHIA[3], G. COEN[3], M. TACCONE GALLUCCI[4], G. SIMONETTI[5], M.R. DESSÌ[6], I. NOFRONI[7], G. SPLENDIANI[3]
Dep. of Nephrology S. Giovanni Addolorata Hospital[1], G.B. Grassi Hospital, Ostia[2], 'Tor Vergata' University[3], Policlinico Casilino[4], Dep. of Radiology, 'Tor Vergata' University[5]; Dep of Laboratory Med. 'Tor Vergata' University[6]; Dep. Experimental Med. and Pathology[7]: 'La Sapienza' University; Rome, Italy

Cardiac calcifications are a frequent finding in uremic patients and probably responsible of fatal heart events. Low levels of AHSG protein (Fetuin-A), an acute negative reactive protein synthesized in the liver, have been considered as a risk factor of cardiac death in dialysis patients. In addition Fetuin-A and Matrix GLA protein complex are able to keep in solution calcium phosphate, inhibiting its precipitation in soft tissues. This study has been carried out in order to evaluate the risk factors of cardiac calcifications and the role of fetuin-A levels in hemodialysis patients. 120 patients on maintenance hemodialysis, age 59.1 ± 13.1 years, HD age 63.5 ± 61.8 months, M/F 77/43, were submitted to evaluation of a cardiac calcification score with fast Multilayer Computerized Tomography (MCT). Moreover calcium phosphate metabolism, lipid and inflammatory parameters, among which PINI index, Fetuin-A and PCR were measured as well as hemoglobin concentration. More than 80% patients had frankly pathologic Score values. Fetuin-A was much lower than normal (0.328 ± 0.11 g/L; NV 0.519 ± 0.15 g/L). Multiregression analysis (MRA) showed selected age (p < 0.0001), serum Cholesterol (<0.0001), Calcium (<0.003), Triglycerides (<0.033) and HDL (<0.059) as variables predictive of log calcium Score, while Fetuin-A was excluded. In addition the coronary calcium Score was correlated to calcitriol IV administration (p < 0.001). Prealbumin was correlated to Fetuin-A in the MRA, while CPR and PINI index were among the excluded variables. In addition Fetuin-A was predictive of Hb concentration. In conclusion there is no indication of a direct involvement of serum Fetuin-A levels in inducing cardiac calcifications, which were mainly linked to age, serum calcium and lipid profile. Fetuin-A seem to be linked to inflammatory and nutritional parameters known to affect Hb concentration and atherogenesis. Fetuin-A, as a cause of cardiac disease does not seem to act through enhancement of arterial calcifications.

W-PO40048

CARDIAC ABNORMALITIES IN DIFFERENT STAGES OF CHRONIC KIDNEY DISEASE

MING-CHENG WANG[1], MENG-FU CHENG[2], AN-BANG WU[2], JENG-JONG HUANG[2], WEI-CHUAN TSAI[3], JU-YI CHEN[3]
Department of Internal Medicine, National Cheng Kung University Hosptial, Tainan, Taiwan[1], Division of Nephrology, Department of Internal Medicine, National Cheng Kung University Hospital[2], Division of Cardiology, Department of Internal Medicine, National Cheng Kung University Hospital[3]

Background: There is a high prevalence of cardiovascular morbidity and mortality in patients with end-stage renal disease, and increased arterial stiffness is an important contributor. However, the relationship among left ventricular (LV) hypertrophy, arterial stiffness and renal function has not been elucidated. We evaluated the cardiac function of patients with CKD in different stages and sought to identify the associated risk factors.
Patients and Methods: In this cross-sectional study, data of 94 patients (59 men and 35 women) with CKD was analyzed. Aortic PWV and cardiac parameters of LV were evaluated. LV mass was calculated from the formula reported by Deverux et al, and was corrected for body surface area to give the LV mass index (LVMI). Univariate and multivariate analysis were performed to obtain the independent factors associated with LVMI.
Results: There was a significant trend for increase in LVMI with the advance of CKD from stage 3 to stage 5 ($P < 0.0001$). Patients with CKD stage 5 have a greater interventricular septal thickness and a lower ejection fraction than those with CKD stage 1–2 and stage 3–4. Univariate linear regression analysis revealed that age, prior cardiovascular disease, diabetes mellitus, hypertension, systolic blood pressure, hemoglobin, serum albumin, calcium, estimated GFR per 1.73 m^2, and PWV correlated with LVMI. In a multivariate model estimated GFR per 1.73 m^2 was an independent determinant of LVMI in patients with CKD (model $R^2 = 0.657$, $P = 0.0236$).
Conclusions: This is the first study to evaluate serial changes of cardiac function in patients with different stages of CKD. There is a stepwise increase in LVMI corresponding with the advance of CKD from stage 3 to stage 5. We also demonstrate that decreased estimated GFR per 1.73 m^2 is independently associated with increased LVMI in CKD patients.

W-PO40049

RELATION OF PULSE PRESSURE WITH ARTERIAL CALCIFICATION AND CAROTID ATHEROSCLEROSIS

ALAATTIN YILDIZ[1], ESAT MEMIŞOGLU[2], HALIL YAZICI[3], AHMET V. CELIK[3], HAMDI PUSUROGLU[3], HUSEYIN OFLAZ[4], YILMAZ NISANCI[4], SAVAS TEPE[5]
Istanbul School of Medicine, Nephrology[1], TEST Tani merkezi[2], Nephrology[3], Cardıology[4], Radiology[5]

Pulse pressure is independent predictor of cardiovascular mortality in hemodialysis (HD) patients. Pulse pressure represents arterial stiffness and function of the large conduit arteries. In this study, we aimed to investigate association between pulse pressure and vascular structural changes such as atherosclerosis and arterial calcifications in HD patients.
Patients and Methods: 108 chronic hemodialysis patients (49 male, 59 female, mean age: 46 ± 13 years) were included. Biochemical analysis, echocardiographic examination and high-resolution carotid Doppler examinations were done. Aortic wall calcification was measured with electron beam computed tomography. Degree of carotid artery stenosis was measured from four different sites (communis, bulbus, interna and externa) in both of the carotid arteries. Carotid plaque scores were calculated by summing the degree of stenosis measured from all locations.
Results: Pulse pressure was correlated with left ventricular mass index (r: 0.58), aortic wall calcification score (r: 0.26) and carotid plaque score (r: 0.27). Patients with carotid plaque (n: 60) had higher pulse pressure than patients without plaque (n: 44) (50 ± 16 mmHg vs 44 ± 14 mmHg, p = 0.05). Patients were divided three groups according to aortic wall calcification score. Pulse pressure was significantly higher in patients with higher aortic wall calcification (54 ± 16 mmHg) than patients with lower aortic wall calcification (44 ± 15 mmHg, p = 0.04).

Pulse pressure was associated with large vessel calcification and extracoronary atherosclerosis. Pulse pressure seems to be a hemodinamic marker of vascular structural changes which are related to cardiovascular mortality in HD patients.

W-PO40050

LEFT VENTRICLE HYPERTROPHY IN HEMODIALYSED PATIENTS

SVETISLAV KOSTIC[1], GORAN PAUNOVIC[1], NEVENA KARANOVIC[1], SONJA SALINGER[1], RADOMIR RAICEVIC[1], KAROLINA PAUNOVIC[1], RADE CUKURANOVIC[1]

Institute of Nephrology and Hemodialysis, Clinic of Cardiovascular Diseases[1]

Introduction: Left ventricle hypertrophy (LVH) is a common cardiac alteration in the terminal stadium of the chronical renal failure (CRF) and one of the predictors of the cardiac mortality in hemodyalised patients. Although the hypertension is considered the main risk factor, LVH is developing and progressing during the time spent on hemodyalises with a weak correlation with the blood pressure. **The aim of the work**: in this work we tried to precisely determine the scope, type and degree of LV hypertrophy in the patients undergoing hemodialysis.

Patients and Methods: Twenty five patients were included in this examination, 12 men and 13 women with an average age of 49.41 ± 11.66. The average length of the dialyses treatment was 55.36 ± 37.23 months. Ehocardiografphically the measurements were made in 2D parasternal long-axis section in end-diastola and end-systole according to the American Society o Cardiology criteria and each parameter was obtained as a value from three heart cycles. The following left ventricle (LF) average parameters were measured: internal diameter in diastole (EDD) and systole (ESD), thickness of the interventricle septum in diastole (IVSd) and the thickness of the posterior wall in diastole (PWd).

Results: Twenty one patients (84%), according to the results obtained, had the changed left ventricle.

The normal LV posterior wall thickness was noticed in 9 patients (36%). Eccentric non-dilatation hypertrophy of LV occurred in four (16%) and eccentric dilatation in 14 patients (56%). LV dilatation in the absence of hypertrophy was exocardiographically found out in one patients (4%).

Conclusion: The most usual change of left ventricular hypertrophy was eccentric dilatational hypertrophy. The degree of hypertrophy was different: from very mild to severe forms. These changes lead to severe rhytm disorders which are the most frequent cause of death in patients undergoing hemodialysis.

W-PO40051

CLINICAL SIGNIFICANCE OF B-TYPE NATRIURETIC PEPTIDE LEVELS AND IMPEDANCE CARDIOGRAPHY IN MAINTENANCE HEMODIALYSIS PATIENTS

BYOUNG GEUN HAN[1], SEUNG OK CHOI[1]

Department of Internal Medicine, Yonsei University Wonju College of Medicine[1]

The risk for cardiovascular disease (CVD) morbidity and mortality is higher in hemodialysis (HD) patients than in general population. Earlier diagnosis, treatment and prevention of CVD are the best way to reduce the single most important cause of death. However, cardiac geometric and/or functional alterations including left ventricular hypertrophy, atherosclerosis and/or systolic and diastolic dysfunction are not able to be easily known to nephrologist in the sense that diagnostic procedure is limited because cardiac angiography and echocardiography are frequently needed. To evaluate the cardiac alteration by noninvasive tools, we measured pre-HD and post-HD B-type natriuretic peptide levels and performed impedance cardiography (ICG) in 40 HD patients.

Pre-HD BNP level, post-HD BNP level, cardiac index (CI), cardiac output (CO), stroke volume (SV), systemic vascular resistance index (SVRI), systemic vascular resistance (SVR), acceleration index (ACI), velocity index (VI) and thoracic fluid content (TFC) in patients were significantly higher than those in normal control group ($p < 0.05$). Pre-HD BNP level, stroke index (SI), SV, TFC were significantly different after HD measurements ($p < 0.05$). There was significant difference of pre-HD BNP level, SI, SV and VI between diabetes group and non-diabetes group ($p < 0.05$). Pre-HD BNP level was correlated significantly with post-HD BNP level, systolic blood pressure, diastolic blood pressure, CO, SVRI, SVR, TFC ($P < 0.05$).

In multiple linear regression analysis, SVR and TFC were positively associated with pre-HD BNP level ($R^2 = 0.289$). The area under the ROC curve for cardiac alterations was 0.749 for pre-HD BNP level. A cut-point of 560 pg/mL for pre-HD BNP level was 80% sensitive and 72% specific in determining cardiac alterations. Even though cardiac alterations of patients were heterogeneous in our study, noninvasive test, plasma BNP level and some parameters (SVR, TFC) of ICG, seem to be available to nephrologist for detecting and monitoring cardiac conditions in HD patients.

W-PO40052

RELATIONS BETWEEN ANTIHYPERTENSIVE TREATMENT EFFICACY, ANTHROPOMETRIC PARAMETERS, AND BLOOD PRESSURE CHANGES DURING HEMODIALYSIS SESSION

MICHAL NOWICKI[1], EWA RUTKOWSKA-MAJEWSKA[2], SYLWIA ZAMOJSKA[2], MAGDALENA SZKLAREK[2]

Dept. Nephrology, Hypertension and Kidney Transplantation, Medical University of Lodz, Poland[1]

Treatment of hypertension in dialysis patients is challeneging because of the frequent occurrence of a phenomenon of combined interdialytic hypertension and intradialytic hypotension. Furthermore, no specific guidelines on antihypertensive treatment in this population has been issued worldwide.

We studied the efficacy of antihypertensive treatment in chronic dialysis patients with relation to anthropometric parameters and intradialytic BP changes.

54 patients from a single dialysis unit chronically receiving antihypertensive treatment (24M, 30F, age 59 ± 13 yrs) were included. A detailed antihypertensive history was taken and BP was measured before and after a mid-week dialysis for three consecutive weeks. Also predialysis basic anthropometrics, body composition with multi-frequency phase-sensitive bioimpedance (NutriGuard M, Data Input, Darmstadt, Germany), hsCRP, hematocrit and serum albumin were measured.

Most of the patients required combined antihypertensive treatment (43 patients received 2 or more drugs). Despite the treatment BP over 130/80 mmHg before and after dialysis was found in 31 and 21 patients, respectively. Interestingly the largest BP decreases during dialysis were found only in those patients with predialysis BP over 140/90 mmHg. The intradialytic decrease of blood pressure during dialysis correlated with interdialytic weight gain ($R = -0.77$, $p < 0.001$), predialysis systolic $R = -0.53$, $p < 0.001$ and diastolic BP $R = -0.39$, $p < 0.001$ and also with total body water, lean body mass and extracellular volume. No relation was found between predialysis BP and its decrease during dialysis, and hematocrit, epoetin dose, CRP, and Kt/V, respectively. In a multivariate analysis the treatment with specific antihypertensive drug classes showed no relation to the control of hypertension, intradialytic blood pressure changes and interdialytic weight gain.

In conclusion, appropriate control of interdialytic hypertension appears to protect from intradialytic blood pressure decreases but the choice of specific antihypertensive agents may not play a role in this respect. Other important risk factors for intradialytic hypotension remain overhydration and low lean body mass.

W-PO40053

ADIPONECTIN IS ASSOCIATED WITH ATHEROSCLEROTIC CARDIOVASCULAR DISEASE IN CAPD PATIENTS

ZANZE YU[1], HAO-HUI NI[1], JIA-QI QIAN[1], LE-YI GU[1], AI-WU LIN[1], WEI FANG[1], LI-OU CAO[1], HUI-LI DAI[1]

Renal Division, Renji Hospital, Shanghai Second Medical University[1]

Background: Adiponectin (Acrp30) is a physiologically active polypeptide hormone secreted by adipose tissue that shows insulin-sensitizing, antiinflammatory, and antiatherogenic properties. The plasma concentrations of ADPN are reduced in patients with coronary artery diseases. It also predicts cardiovascular death in patients undergoing hemodialysis. Peritoneal dialysis patients are particularly prone to atherosclerosis due to additional glucose load into the peritoneum, hyperinsulinemia and hypoalbuminemia. The present research aimed to investigate the relationship between ADPN and the development of atherosclerotic cardiovascular diseases in CAPD patients.

Methods: We dectected the serum concentration of ADPN by using ELISA and estimated the relationship between ADPN and lipid level, inflammation parameters (CRP, fibrinogen, D-dimer), intima-media thickness (IMT), carotid plaque and the history of cardiovascular diseases (CVD) in 59 CAPD patients.

Results: Serum ADPN levels were 2 times higher ($P < 0.01$) among PD patients (13.09 ± 7.54 μg/ml) than among healthy subjects (6.65 ± 4.33 μg/ml). The concentrations of serum ADPN were inversely related to age, BMI, triglycerides,

lnCRP and directly related to HDL in CAPD patients. Compared with patients without carotid plaques the ADPN levels were lower ($P < 0.01$) in patients with plaques (15.17 ± 7.48 μg/ml vs 9.60 ± 5.09 μg/ml). Serum ADPN levels were lower ($P < 0.05$) among patients with atherosclerotic cardiovascular disease (8.68 ± 4.65 μg/ml) than other patients (14.33 ± 7.67 μg/ml). The correlation between ADPN levels and weekly CCr, Kt/V, residual glomerular filtration rate were not significant. The peritoneum membrane transport type or the glucose concentration of dialysate did not appear to affect the levels of ADPN.
Conclusion: The results show that ADPN is correlated with dyslipidemia, inflammation, and atherosclerosis in PD patients. These relations are in a manner consistent with the hypothesis that this protein may act as a protective factor for the cardiovascular system in PD patients.

W-PO40054

EVALUATION OF EFFECT OF ERYTHROPOIETIN AND VIT E ON CARDIAC FINDING OF HEMODIALYSIS PATIENTS

SIMIN SADEGHI BOJD[1]
Zahedan University[1]

Cardiovascular complications are the leading cause of morbidity and mortality in hemodialysis patients. Cardiomyopathy in these patients are multifactorial and various therapeutic options can be considered. The aim of study was to evaluate effect of vitamine E on cardiac system by Electrocardiograghic and echocardiographic finding.
Methods and Material: Twenty five children and adolescents with ESRD were studied. At first they examed and ECG and echocardiography M MODE and Doppler was done. They treated with Erythropoeitin for 3 month and after a break peri.
Results: In this study the effect of combination therapy in cardiac function was better Erythropoeitin. Ventricular mass and ventricular septum thickness decreased. In Wilcoxon signed Rank test (P < 0/025) improvement of LA in combination therapy was significant (P = 0/013) and septal thickness in diastolic period decreased (P = 0/019). Result of Doppler echocardioghrapy studid with Spearman test. There was any coeffient correlation between E/A tricuspid and pulmonary velocity. E/A mitral valve was correlated with ejection fraction and shortening fraction of left ventricular mass. (P = 0/02) Summary of resultsis in the following table:

Response	Erythropoietin	Erythropoietin and vitE (P. Value)
left Atrium	0/22	0/013
End Diastolic septal thickness	0/42	0/019
Left ventricular mass	0/02	0/018

W-PO40055

LEFT VENTRICLE ECHOCARDIOGRAPHIC PARAMETERS AND CARDIAC HYPERTROPHY

BEATA BZOMA[1], BOLESLAW RUTKOWSKI[1], ALICJA DEBSKA-SLIZIEN[1], MARIA DUDZIAK[2]
Department of Nephrology Medical University of Gdansk[1]*, Department of Noninvasive Cardiologic Diagnosis*[2]

Introduction: Left ventricular hypertrophy (LVH) is the main manifestation of uraemic cardiomyopathy and predicts both cardiovascular events independently on conventional risk factors. The irregular activity of renin-angiotensin-aldosteron system is to be a potential causal factor of cardiovascular damage. We investigated the association between Angiotensin Converting Enzyme I/D (ACE I/D), Angiotensinogen M235T (AGT M/T), Receptor AT1 A1166C (AT1 A/C), Aldosterone Synthase CYP11B T(-344)C (CYP11B T/C) gene polymorphism and left ventricle (LV) echocardiographic parameters and the prevalence of LVH in HD patients.
Methods: LV diastolic dimension (LVDD), LV systolic dimension (LVDS), posterior wall thickness (PWT), interventricular septal thickness (IVS) and left ventricular mass index (LVMI) were assessed by echocardiography in 203 patients treated with maintenance HD. LVH was defined for >108 g/m^2 in female and 118 g/m^2 in male. Genomic DNA was amplified by the polymerase chain reaction, followed by restriction enzyme analysis. The Hardy-Weinberg equilibrium was tested by exact tests. Differences in mean values of echocardiographic parameters between genetic groups were tested with test U Manna Witneya.

Results: LVH was present in 171 (84%) patients and 145 (71%) patients were hypertensive. The frequency of genotype DD in a group with LVH was higher than expected from Hardy-Weinberg equation (HW model $N_\alpha = 187$; n = 191), but we did not find differences in LVMI value in patients in different genotypes ACE I/D polymorphism. Polymorphism AGT M/T and CYP11B T/C were not associated with the LVMI value in our patients. LVMI, LVDD, LVDS mean values were significantly higher in a CC than AA AT1 group. There were no differences in LVDD, LVDS, PWT and IVS values in different genetic groups of polymorphism I/D ACE, M/T AGT and C/T CYP11B2.
Conclusions: Genotype CC AT1 receptor gene could be a contributory factor for the development of LV hypertrophy and dilatation in HD patients.

W-PO40056

NON-INVASIVE EVALUATION OF CARDIOVASCULAR DISEASE (CVD) IN YOUNG ADULTS WITH CHILDHOOD-ONSET CHRONIC KIDNEY DISEASE

UWE QUERFELD[1], SONIA BRIESE[1], SUSANNE WIESENER[1], JOACHIM WILL[1], DIETER HAFFNER[1]
Pediatric Nephrology, Charité University Hospital, Berlin, Germany[1]

Background: We studied 40 adult patients (aged 21.7 +/– 5.5 years) who developed ESRD at the age of 11.5 +/– 4 years (cumulative time on dialysis 2.9 +/– 3.5 years) and 40 matched healthy control subjects. At the time of investigation, 9 patients were on dialysis and 31 had a functioning kidney transplant. Non-invasive diganostic methods included carotid artery intima-media thickness (IMT), electron beam computed tomography (EBCT), echocardiography and venous occlusion plethysmography. Results of these measurements were used as surrogate parameters of atherosclerosis and correlated with measured levels of traditional and non-traditional risk factos and the cumulative amount of prescribed medication.
Results: The mean survival of all patients treated for ESRD at the Charité Children's Hospital and not lost to follow-up (n = 190) was 30.6 years, the cardiovascular mortality 33%. IMT was not significanly different in patients and controls; 4 of 40 patients (10%) had coronary calcifications on EBCT. Echocardiography showed that 62.5% of patients had left ventricular hypertrophy. Patients had a 40% reduction of post-ischemic blood flow and post-ischemic peak flow. Morphological alterations of the heart and arteries were significantly correlated with the duration of ESRD and dialysis time, and with the cumulative intake of calcium from phosphate binders and active vitamin D preparations, but not of immunosuppressive drugs. Functional changes (vascular reactivity) were correlated with non-traditional risk factors.
Conclusions: Young adults with ESRD since childhood show a distinct pattern of cardiovascular damage characterized by a decrease in vascular reactivity, the occurrence of coronary artery calcifications and changes in left ventricular morphology. It is likely that these systemic alterations represent an 'early' phenotype in the development of uremic CVD. Calcium-containing phosphate binders and active vitamin D preparations are significantly involved in the pathogenesis.

W-PO40057

VASCULAR STIFFNESS DURING ORTHOSTATISM IN HEALTHY CONTROLS WITH PATIENTS WITH RENAL FAILURE

MARTIN TEPEL[1], ALEXANDRA SCHOLZE[1], ALEXANDRA MAIER[1], FRANZISKA STOCKS[1]
Med Klinik IV, Charite Campus Benjamin Franklin, Germany[1]

Vascular stiffness during orthostatism in healthy controls with patients with renal failure.
Increased sympathetic activity has been linked to hypertension and increased cardiovascular morbidity. However, non-invasive measurements of vascular stiffness after activation of sympathetic activity are sparse. Now we used radial artery waveform analysis obtained with a calibrated tonometer for evaluation of reflective arterial stiffness of small arteries during orthostatic tests in 16 healthy subjects. Radial artery waveforms were obtained using a calibrated tonometer (model CR-2000, Hypertension Diagnostics Inc., Minn., USA) applied to the patients' radial artery at the wrist overlying the radial bone prominence. Arterial waveforms were calibrated to the systolic and diastolic cuff pressures. A computer-based third order four-element Windkessel model of the circulation was used to match a diastolic pressure decay of the waveforms and quantify changes in terms of small artery reflective arterial stiffness. Measurements were performed during a standardized orthostatic test.

During the orthostatic test the heart rate significantly increased from 71 ± 10/Min to 90 ± 12/Min (mean ± SD, $p < 0.001$), indicating sympathetic nerve activation. During the orthostatic test the small artery reflective arterial stiffness significantly increased from 10.80 ± 2.99 mmHg/mL to 14.47 ± 5.55 mmHg/mL ($p < 0.05$). Reflective arterial stiffness significantly correlated with the heart rate ($r^2 = 0.32$, $p = 0.0002$). In addition, systolic blood pressure, diastolic blood pressure and systemic vascular resistance increased significantly during the orthostatic test (systolic blood pressure from 121 ± 12 mmHg to 127 ± 17 mmHg; diastolic blood pressure from 67 ± 10 mmHg to 77 ± 10 mmHg; systemic vascular resistance from 1131 ± 235 dyn * sec * cm-5 to 1515 ± 342 dyn * sec * cm-5; each $p < 0.001$). Reflective arterial stiffness increases during orthostatic tests probably due to sympathetic activity. Non-invasive measurement of reflective arterial stiffness can therefore help to evaluate the role of sympathetic activation in the pathophysiology of vascular responses in healty controls and patients with chronic renal failure.

W-PO40058

SHORT TERM VARIABILITY OF BNP AND NT-PROBNP IN HEMODIALYSIS PATIENTS

STEPHANIE GRAF[1], SHARZAD ASHAYER[2], WINFRIED FASSBINDER[1], HARALD RENZ[2], HANS GUENTHER WAHL[2]
Internal Medicine and Nephrology, Hospital of Fulda, Germany[1], Clinical Chemistry and Molecular Diagnostics, Philipps University of Marburg, Germany[2]

B-type natriuretic peptide (BNP) and NT-proBNP, the biologically inactive N-terminal fragment of proBNP, are suggested as cardiac biomarkers in diagnosis and treatment of chronic heart failure. Both forms can be elevated in patients with chronic kidney disease, but only few data are available in hemodialysis (HD) patients. Short term interdialytic changes of BNP and NT-proBNP were studied in HD patients.
BNP (ADVIA BNP assay, Bayer) and NT-proBNP (Elecsys proBNP, Roche Diagnostics) were drawn from 27 HD patients (16 men, 11 women, age 68.4 ± 11.7 years, HD vintage 5.7 ± 3.2 years, mean ± SD) on six consecutive HD sessions. In addition, corresponding clinical data were analyzed.
Plasma concentrations of BNP were elevated in most patients (n = 24, day 1: 666 ± 183 pg/ml; mean ± SEM). All patients had clearly elevated NT-proBNP values (day 1: 20297 ± 5562 pg/ml). Changes were calculated relative to the first (mid-week dialysis) values. BNP values showed a minimum change of 6% and a maximum change of 94% after the two-day interdialytic interval. NT-proBNP values exhibited only small changes independent of the length of the interdialytic interval (changes from baseline value between –15% and +9%). Analyzing the clinical data, there was a positive correlation for BNP with the change of ultra filtration volume (BNP versus UF: range from 0.23 to 0.96). The correlation was much weaker for NT-proBNP (NT-proBNP versus UF: range from –0.49 to 0.94). Correlation between BNP/UF and NT-proBNP/UF was 0.77.
In hemodialysis patients, BNP and NT-proBNP are clearly elevated. BNP exhibits a greater interdialytic variability. As both forms are secreted in equimolar amounts by the left ventricle, a higher clearance of BNP through dialysis is probable. A positive correlation between the rate of weight change and BNP values (weaker for NT-proBNP) can be found in most patients and may reflect volume status.

W-PO40059

BNP AND NT-PROBNP AS DIAGNOSTIC AND PROGNOSTIC MARKERS IN HEMODIALYSIS PATIENTS

STEPHANIE GRAF[1], SHARZAD ASHAYER[2], MOHAMMED ATRISSI[1], WINFRIED FASSBINDER[1], HARALD RENZ[2], HANS GUENTHER WAHL[2]
Internal Medicine and Nephrology, Hospital of Fulda, Germany[1], Clinical Chemistry and Molecular Diagnostics, Philipps University of Marburg, Germany[2]

B-type natriuretic peptide (BNP) and NT-proBNP are suggested as cardiac biomarkers in the diagnosis and treatment of chronic heart failure. Both forms can be elevated in patients with chronic kidney disease, who often suffer from cardiovascular related diseases and heart failure. The diagnostic and prognostic value of both parameters was analyzed.
BNP (ADVIA BNP, Bayer) and NT-proBNP (Elecsys proBNP, Roche Diagnostics) were drawn from 131 chronic hemodialysis (HD) patients (71 men, 60 women, age 67.8 ± 12.2 years, HD vintage 5.1 ± 4.1 years, mean ± SD) after a two-day interdialytic interval. As clinical data ultra filtration (UF) rates and mean arterial pressure (MAP) were recorded. For 45 patients echocardiographic data were available. All patients were followed up for 1 year recording all deaths and cause of death.
Nearly all patients showed elevated levels of BNP (1056.6 ± 129.8 pg/ml, mean ± SEM) and NT-proBNP (26126 ± 4255 pg/ml). No correlation was found for mean UF rates (BNPvsUF: –0.03; NT-proBNPvsUF: –0.02) and MAP values (BNPvsMAP: –0.03; NT-proBNPvsMAP: 0.06). Positive correlations were documented for left ventricular mass index (LVMI) and an inverse correlation for ejection fraction (EF): BNPvsLVMI: r = 0.38; NT-proBNPvsLVMI: r = 0.30; BNPvsEF: r = –0.31; NT-proBNPvsEF: r = –0.29. 26 deaths occurred and 17 deaths were classified as cardiovascular. Dividing the cohort into 3 groups according to BNP and NT-proBNP values, there was a trend for higher mortality with high BNP and NT-proBNP values (BNP: 1st group: 0–310 pg/ml: 7 deaths/3 CV; 2nd group: 310–900 pg/ml: 7/6; 3rd group: 901–8400 pg/ml: 12/8; NT-proBNP: 1st group: 0–4.870 pg/ml: 4/3; 2nd group: 4.870–15.300 pg/ml: 9/5; 3rd group: 15.300–345.000 pg/ml: 13/9).
In hemodialysis patients, both natriuretic peptides are clearly elevated. LVMI and EF correlated with BNP and NT-proBNP values. High values of natriuretic peptides were associated with higher cardiovascular mortality.

W-PO40060

CORONARY ARTERY DISEASE IN SYMPTOMATIC AND ASYMPTOMATIC PATIENTS ON HEMODIALYSIS

MARIA WANIC-KOSSOWSKA[1], JULIA POPOWSKA-DROJECKA[2], MIKOLAJ KOBELSKI[1], ANDRZEJ OKO[1], LIDIA KOZIOL[1], ELZBIETA PAWLICZAK[1], MAREK MUSZYTOWSKI[2], STANISLAW CZEKALSKI[1]
Department of Nephrology, Transplantology and Internal Diseases, Poznan University of Medical Sciences, Poland[1], Nephrology Department, Central Hospital Torun, Poland[2]

All patients on hemodialysis (HD) should be considered to be at risk of coronary artery disease, which is sufficient enough to warrant aggressive diagnosis and management. Coronary angiography (CA) was suggested as essential for evaluating coronary disease in uremic patients since the diagnosis of the disease based only on chest symptoms and clinical factors proved to be difficult. Convincing evidence is available showing the impact of coronary artery disease on patient survival both on HD and after kidney transplantation. Some investigators suggested to screen patients by CA before renal transplantation, even when the patients were without symptoms and clinical factors of coronary artery disease.
The aim of this study was to compare the prevalence of coronary artery disease evaluated by CA in 4 symptomatic patients (2M, 2F, aged 61.8+/–12.7 years) and 13 asymptomatic patients (9M, 4F, aged 56.3+/–12.3 years) treated by HD and prepared for kidney transplantation. In all patients echocardiography and troponin I (cTnI) serum levels were evaluated.
In the group of symptomatic patients: 1 had substantial coronary artery calcification and in 3 patients critical coronary arteries narrowing was found. Mean left ventricular mass (LVM) was 229.5+/–43.0 g and cTnI serum level was 0.28+/–0.22 ng/mL. Seven asymptomatic patients had massive coronary arteries calcifications with critical coronary arteries narrowing in 4 of them. Remaining 2 patients had microvascular changes. In this asymptomatic group mean LVM was 269.5+/–66.4 g and cTnI serum level was 0.27+/–0.24 ng/mL. Mean LVM in the group of asymptomatic patients was greater in comparison with symptomatic patients, however this difference was not significant as well as the difference in mean serum cTnI level.
Conclusion: High incidence of advanced coronary artery disease in asymptomatic HD patients was confirmed. Neither LVM nor cTnI level seem useful for suspicion of advanced coronary artery changes. The patients prepared for renal transplantation should undergo CA irrespectively of coronary disease symptoms.

W-PO40061

PROGNOSTIC IMPLICATION OF LEFT VENTRICULAR HYPERTROPHY IN CHRONIC HEMODIALYSIS PATIENTS AND REDUCTION OF LEFT VENTRICULAR MASS BY ACE INHIBITOR – COMPARATIVE STUDY OF IMIDAPRIL AND CANDESARTAN

TOSHIHIKO ISHIMITSU[1], NOBUKO MATSUMOTO[1], ATSUSHI OKAMURA[1], HITOSHI SETA[1], JUNICHI MINAMI[1], HIDEHIKO ONO[1], HIROAKI MATSUOKA[1]
Department of Hypertension and Cardiorenal Medicine, Dokkyo University School of Medicine, Japan[1]

We examined the influence of left ventricular hypertrophy (LVH) on the prognosis of hemodialysis patients and compared the effects of ACE inhibitor and angiotensin II receptor blocker (ARB) on left ventricular mass (LVM). When 551 chronic hemodialysis patients were followed for 5 years, 67 out of 153 patients (43.8%) showing LVH on electrocardiogram died, while 107 out of 396 patients (27.0%) without LVH died (chi square = 14.3, $p < 0.001$). Fatal and non-fatal cardiovascular events occurred in 80 patients (52.3%) with LVH and in 123 patients (31.1%) without LVH (chi square = 21.3, $p < 0.001$). Then, 45 patients on chronic hemodialysis were divided into 3 groups of 15 patients each and given placebo (P), 2.5 mg imidapril (IM) or 4 mg candesartan (CS) once daily for 6 months. Before and after the 6-month period, LVM was evaluated by echocardiography and circulating factors of renin-angiotensin system were measured. Systolic blood pressure was lowered by 11 and 10 mmHg after 2 and 3 months in the CS group, but was not significantly changed in the P and IM groups. In CS, plasma AII was increased after 6 months. In IM, serum ACE was reduced and plasma renin activity was increased, however, plasma AII was not significantly changed. LVM index was significantly decreased in IM (132+/–10 to 109+/–6 g/m^2, $p = 0.04$) but was unchanged in P (129+/–6 to 126+/–5 g/m^2) or CS (130+/–9 to 125+/–7 g/m^2). It is suggested that LVH is a strong prognostic factor for the incidence of death and cardiovascular events and an ACE inhibitor reduces LVM by the mechanism independent of blood pressure changes in hemodialysis patients.

W-PO40062

RELATIONSHIP OF CORONARY ARTERY BYPASS GRAFTING (CABG) OUTCOME TO DIFFERENT STAGES OF PRE-OPERATIVE MILD TO MODERATE CHRONIC KIDNEY DISEASE (CKD)

ATASHA ASMAT[1], CHEE KHUN HO[2], POO SING WONG[1], MARK DA COSTA[1], CHRISTIE TAN[1], CHUEN NENG LEE[1], TITUS W LAU[2]
Department of Cardiothorasic Surgery, National University Hospital, Singapore[1], Department of Medicine, National University Hospital, Singapore[2]

The aim of this study was to define CABG outcome according to the stage of CKD at the time of surgery.

All consecutive on-pump CABG done in our institution from April 2003 to March 2004 were included. Data were collected prospectively. Stage of CKD was assigned according to serum creatinine taken within 48 hours before surgery. Glomerular filtration rate (GFR) was estimated by Cockcroft-Gault formula. Stages of CKD were defined according to the NKF K/DOQI guidelines and outcome of interest were 30-day all-cause mortality and sum of peri-operative complications. Stages 4–5 CKD were excluded. Chi-square test and one-way ANOVA were used.

Results: A total of 253 patients were included with a mean age of 59.5 ± 8.8 years. There were 73 patients with normal GFR (^{3}90 ml/min), 126 patients in Stage II (GFR of 60–89 ml/min) and 54 patients in Stage III CKD (of 30–59 ml/min) respectively. Age was inversely correlated with stage of CKD. The groups did not significantly differ in their pre-op left ventricular ejection fraction, number of grafting, diabetic status and the elective or emergent nature of CABG. There were 10 deaths in total (1 in normal GFR, 6 in Stage II and 3 in Stage III). The mean number of complications were 0.4 ± 0.62 for the normal GFR group, 0.67 ± 0.95 for Stage II and 0.89 ± 1.09 for Stage III. There were significantly more complications in Stage III group as compared the normal GFR group ($p = 0.008$). There was no difference in the all-cause mortality rate between the groups. Age was not significantly correlated with mortality or sum of peri-operative complications.

Conclusion: Stage III CKD is associated with a greater peri-operative complication rate than patient with a normal GFR. However, there was no significant mortality difference between the two groups.

Renal Osteodystrophy – Phosphate and Calcium

W-PO40063

GLOMERULAR FILTRATION RATE DEPENDENT RELATION OF ARTERIOSCLEROSIS WITH BONE MINERAL DENSITY IN POSTMENOPAUSAL WOMEN WITH RENAL INSUFFICIENCY

NOBUHIKO JOKI[1], HIROKI HASE[1], YURI TANAKA[1], YASUNORI TAKAHASHI[1], HIROYASU ISHIKAWA[1], YOJI INISHI[1], TOMOKATSU SAIJYO[2], YOSHIHIKO IMAMURA[2]
Division of Cardiology & Nephrology, Toho University Ohashi Hospital[1], Division of Dialysis Center, Nissan Tamagawa Hospital[2]

Background: It is recently revealed that bone mineral density (BMD) was inversely associated with arterial stiffness in chronic hemodialysis patients. Our hypothesis is that the inversely relationship between BMD and arterial stiffness may be dependent of the degree of renal function.

Purpose: Our purpose is to clear the difference of intensity on relationship between BMD and arterial stiffness in patients with various renal functions.

Methods: We planed a cross-sectional study. The criteria of enrolling of this study was 1) postmenopausal women, 2) Both BMD of radius by dual-energy X-ray adsorptiometry (DEXA) and pulse wave velocity (PWV) were performed within one year, and 3) calculated GFR below CKD stage 2. Two-hundred post-menopausal women were enrolled of this study. Mean age was 73, and mean calculated GFR was 46 ml/min. Patients were divided three groups according to calculated GFR named CKD stage 2&3, 4, and 5. The clinical characteristics and relation of BMD to PWV were compared between 3 groups.

Results: The distribution of patients was 147, 15, and 38 patients in CKD 2&3, 4, 5 group respectively. Although, in all CKD stage, DEXA score was associated inversely with PWV, the intensity of regression coefficient was stronger in upper level of CKD stage (regression coefficient between volume of Ca (g/cm^2) or Z-score (%) and PWV; –0.148, –0.421, and –0.552 or 0.063, –0.159, and –0.331 in CKD 2&3, 4, 5 groups respectively).

	CKD 2–3	CKD 4	CKD 5
baPWV (m/sec)	19.3 ± 4.3	20.2 ± 4.3	21.5 ± 6.2
DEXA			
Ca (g/cm^2)	0.45 ± 0.10	0.43 ± 0.12	0.46 ± 12
Z-score (%)	102 ± 21	93 ± 22	93 ± 19
T-score (%)	70 ± 16	66 ± 19	71 ± 18
Regression coefficient			
Ca / baPWV	–0.148	–0.424	–0.552
Z / baPWV	0.063	–0.159	–0.331
T / baPWV	–0.148	–0.421	–0.548

Conclusion: The inversely relation between BMD and arterial stiffness is clearly dependent on the degree of renal function. These results suggest that by maintaining of BMD from early stage of CKD, accelerating progression of arteriosclerosis over reducing residual renal function may change to slow down.

W-PO40064

RE-EVALUATION OF SERUM PHOSPHORUS CONTROL AFTER PARATHYROIDECTOMY IN HEMODIALYSIS PATIENTS WITH SECONDARY HYPERPARATHYROIDISM

TAKASHI YOKOYAMA[1], YASUHIRO ONODERA[1], TSUTOMU MANO[1]
Department of Nephrology, Sapporo Tokushukai Hospital[1]

In order to clarify the risks with hyperphosphatemia after Parathyroidectomy (PTx) in hemodialysis (HD) patients with secondary hyperparathyroidism (II° HPT), we retrospectively analyzed 21 cases received PTx (11 males and 10 females, from 35 to 61 years old with an average of 52.12 ± 9.65, HD duration ranging from 32 to 139 months with an average of 67.74 ± 42.16). The levels of serum phosphorus (P), albumin-corrected calcium (Ca), CaxP product (CaxP) and intact parathyroid hormone (PTH) were serially determined for the length of one to 6 years before and after PTx. The levels of PTH, P and CaxP that were calculated just before PTx were 669.90 ± 284.32 pg/mL, 8.93 ± 1.60 mg/dL and 74.73 ± 16.55 mg^2/dL^2, respectively. Excellent reduction greater than 95.0% of initial values of PTH was obtained after PTx in all 21 cases. After operation, a daily dose volume (1.0–9.0 g) of sevelamer hydrochloride (sevelamer) and/or 5–10 μg of intravenous maxacalcitol were prescribed in order to keep P, CaxP and PTH levels within the NKF-K/DOQI guideline range. These parameters have been kept within appropriate ranges in 14 cases out of 21. In the other 7 cases, P, CaxP and PTH levels increased more than 8.0 mg/dL, 55 mg^2/dL^2 and 500 pg/mL, respectively. In five out of the 7, sevelamer and maxacalcitol administrations were effective and the values of P, CaxP and PTH maintained less than 5.5 mg/dL, 55 mg^2/dL^2 and 250 pg/mL, respectively. As the appropriate control of P by sevelmer and the PTH-suppressing effect of maxacalcitol were not sufficient enough to prevent II° HPT in the two cases, the forearm auto-transplantation parathyroid was removed in one patient and residual parathyroid in neck was removed in the other case. These results indicate that it is quite important for HD patients received PTx to maintain appropriate levels of serum P and CaxP in order to prevent occurrence of recurrent II° HPT.

W-PO40065

LONG-TERM SAFETY, EFFICACY, AND TOLERABILITY OF LANTHANUM CARBONATE: RESULTS FROM A 3.5-YEAR STUDY

ALASTAIR HUTCHISON, ON BEHALF OF THE SPD405-301 LANTHANUM STUDY GROUP[1]
Manchester Royal Infirmary[1]

Hyperphosphataemia is a common complication in patients with end-stage renal disease (ESRD). Lanthanum carbonate (LC) is a new phosphate binder that has been shown to effectively reduce serum phosphorus levels in these patients. We present the results of a 6-month open-label extension to a previously reported 3-year study that assessed the safety, efficacy, and tolerability of LC treatment in patients with ESRD.

During the initial phase of study, patients were randomized to receive LC or calcium carbonate for 6 months. All patients then received LC for 6 months and could continue treatment with LC (1500–3000 mg/day) for a further 24 months. Safety, tolerability, and maintenance of serum phosphorus control (≤5.6 mg/dL [1.8 mmol/L]) were assessed. After 3 years of treatment, there was little change in plasma lanthanum levels and no differences in the incidence or types of adverse events during the three individual years of the study. All patients who completed the trial had the option to receive a further 6 months' LC treatment. In total, 51 patients (33 men, 18 women; mean [± SD] age, 54.1 ± 13.3) entered this 6-month extension. After 3.5 years of LC treatment, 53% of patients had phosphorus levels <1.8 mmol/L, the definition of control in the comparator-controlled phase of the study. Mean (±SD) serum phosphorus, calcium, and calcium ′ phosphorus levels, after 3.5 years of treatment were 1.91 ± 0.59 mmol/L, 2.38 ± 0.17 mmol/L, and 4.58 ± 1.51 $mmol^2/L^2$, respectively. Forty-eight (94%) of patients experienced a new adverse event in this 6-month extension, of whom only three (6%) were considered to have a treatment-related adverse event.

W-PO40066

A 2-YEAR RANDOMIZED, COMPARATOR-CONTROLLED TRIAL INVESTIGATING THE EFFECT OF LANTHANUM CARBONATE ON BONE

HARTMUT MALLUCHE, ON BEHALF OF THE SPD405-307 STUDY GROUP[1]
University of Kentucky, Lexington, KY, USA[1]

Hyperphosphatemia in patients with end-stage renal disease (ESRD) is commonly associated with renal osteodystrophy. The effect of the new phosphate binder, lanthanum carbonate (LC), on bone, was investigated during a 2-year comparative study in patients with ESRD.

After a 1–3-week washout phase and double tetracycline labeling, a bone biopsy was taken from the iliac crest for bone histology and histomorphometry. Patients were randomized to receive LC or their pre-study phosphate binder (standard therapy), for 2 years, including a 6-week dose-titration phase. Patients were randomized to receive a second bone biopsy after 1 or 2 years of treatment.

In total, 206 patients were randomized to receive LC ($n = 105$) or standard therapy ($n = 101$); paired baseline and 2-year follow-up biopsies were collected from 38 of these patients (LC, $n = 22$; standard therapy, $n = 16$). After 2 years' treatment there was no difference observed between the treatment groups in mineralization lag time, osteoid thickness, or activation frequency, ($p > 0.1$). In addition, there was no evidence of development of osteomalacia in either group after 2 years of treatment. At the end of the study, median parathyroid hormone levels in the LC and standard therapy groups were 214 ng/L, and 151 ng/L, respectively. Throughout the study, serum phosphorus was similar in the two groups and serum calcium levels were statistically higher in the standard therapy group. Calcium ′ phosphorus product levels were similar in the groups during maintenance treatment.

The findings from this study indicate that 2 years of treatment with LC did not have an adverse effect on bone mineralization, bone turnover, or bone balance in patients with ESRD.

W-PO40067

SAFETY AND TOLERABILITY OF LONG-TERM TREATMENT WITH LANTHANUM CARBONATE

GHODRAT SIAMI[1], WOLFGANG BACKS[2]
VA Medical Center, Vanderbilt University, Nashville, TN, USA[1], Dialysepraxis Barmbek, Hamburg, Germany[2]

The efficacy and tolerability of lanthanum carbonate, a new phosphate binder for the treatment of hyperphosphataemia, have been demonstrated for periods of up to 3 years' continuous treatment in randomized, controlled trials and open-label extensions. We present the results of an additional open-label extension to further assess long-term safety and maintenance of phosphorus control during continued lanthanum carbonate treatment.

Patients who participated in any of four previous studies of lanthanum carbonate and continued to require phosphate binder therapy were eligible to enter this 2-year, open-label extension. Patients were not eligible for this open-label study if they had withdrawn from a previous study before randomization, or were withdrawn as a result of adverse events classed as 'related' or 'possibly related' to study medication.

The safety of lanthanum carbonate was assessed by adverse event recording and by monitoring of laboratory values and vital signs. Adverse events were also assessed according to length of exposure to lanthanum carbonate. Efficacy was assessed as control of pre-dialysis serum phosphorus levels. Control of serum phosphorus was defined as ≤5.9 mg/dL (1.9 mmol/L) for patients previously enrolled in the US studies, and ≤5.6 mg/dL (1.8 mmol/L) for those previously enrolled in the European studies.

We will present data from this trial extension, on the long-term safety, tolerability, and efficacy of lanthanum carbonate in patients who have received up to 5 years of treatment.

W-PO40068

NO ADVERSE EFFECTS ON HAEMATOLOGICAL PARAMETERS DURING LANTHANUM CARBONATE TREATMENT IN OVER 2000 PATIENTS

ALASTAIR HUTCHISON[1], ON BEHALF OF THE LANTHANUM STUDY GROUP[1]
Manchester Royal Infirmary, Manchester, UK[1]

The efficacy and tolerability of lanthanum carbonate have been demonstrated in extensive pre-clinical and clinical trial programmes. We review haematology data for over 2000 dialysis patients treated in four separate clinical trials and an open-label extension period for up to 3 years. Parameters evaluated include serum iron, transferrin, ferritin, folate, vitamin B12, and the usual cellular measurements.

During a randomized, double-blind study with LC ($n = 50$) *vs.* placebo ($n = 44$) no significant difference between treatment groups was seen in any of the parameters assessed ($p > 0.05$). In a second study, (LC, $n = 455$; calcium carbonate [CC], $n = 92$), no significant difference between treatment groups was seen with any parameter after 6 months ($p > 0.05$) except MCV, which showed a significantly greater increase in the CC group ($p < 0.0001$). After 6 months, mean (±SD) MCV was 97.6 ± 6.9 fL in the LC group, compared with 101.5 ± 7.1 fL in the CC group. No significant difference in MCV, or any other haematological parameter assessed, was seen after 1 year of treatment in a third, randomized study of LC ($n = 49$) *vs.* CC ($n = 49$; $p > 0.05$). In a fourth study, comparing LC ($n = 680$) with standard therapy ($n = 674$), no significant between-group differences were seen in any of the parameters after 2 years of treatment ($p > 0.05$). Open-label treatment with LC for up to 3 years led to slight changes in haematological parameters, which were not considered to be clinically significant.

In conclusion, LC did not adversely affect haematological parameters when compared with currently available phosphate binders.

W-PO40069

NO EVIDENCE OF HEPATOXOCITY WITH LANTHANUM CARBONATE – CLINICAL TRIAL DATA FROM 2000 DIALYSIS PATIENTS

ALASTAIR HUTCHISN, ON BEHALF OF THE LANTHANUM STUDY GROUP[1]
Manchester Royal Infirmary, Manchester, UK[1]

During toxicity studies with oral lanthanum carbonate (LC), there were no adverse effects on serum liver markers or histology, in any study. Intravenous lanthanum chloride resulted in mild chronic liver inflammation in dogs given the highest dose (1 mg/kg/day) for 4 weeks. These changes occurred at plasma lanthanum concentrations 20,000x those observed in LC-treated dialysis patients.
In a short-term, randomized, double-blind study with LC ($n = 50$) *vs.* placebo ($n = 44$), no significant changes between treatment groups were observed in alanine transaminase (ALT) or gamma-glutamyl transpeptidase (GGT). In a second study, after 6 months of LC ($n = 455$) or calcium carbonate (CC; $n = 92$), no significant changes were seen in ALT or GGT; increased liver enzymes were considered treatment related in two patients in each group. In a randomized 1-year trial of LC ($n = 49$) and CC ($n = 49$), enzyme levels were similar to those reported in the 6-month study. In a 2-year trial comparing LC ($n = 680$) with standard therapy ($n = 674$), no significant between-group differences were seen in ALT or GGT. Twenty-eight patients (4%) in the LC group and 63 (7%) in the standard therapy group experienced liver adverse events (AEs). In a series of open-label studies providing 3 years of LC, no significant changes were observed in ALT or GGT. In the final 2 years' exposure, three patients (2%) experienced liver-related AEs.
Other than noted above, liver AEs were not considered likely to be related to LC in any study. The lack of hepatotoxicity seen in pre-clinical LC studies is reflected in the lack of effect on liver enzymes and the low level of liver AEs in clinical trials.

W-PO40070

EFFICACY AND TOLERABILITY OF INCREASED DOSES OF LANTHANUM CARBONATE USING A NEW FORMULATION IN PATIENTS WITH ESRD

MAURICE LAVILLE, ON BEHALF OF THE SPD405-313 STUDY GROUP[1]
Université Claude Bernard, Hôpital Edouard Herriot, Lyon, France[1]

Lanthanum carbonate is an effective phosphate-binding agent for the treatment of hyperphosphataemia in patients with end-stage renal disease. Clinical trials have demonstrated the efficacy of lanthanum carbonate using doses of up to 3750 mg/day of elemental lanthanum for up to 3 years. Recently revised guidelines by the Kidney Disease Outcomes Quality Initiative (K/DOQI) recommend that serum phosphorus should be maintained in the range 3.5–5.5 mg/dL (1.13–1.77 mmol/L). In this study, a new formulation of lanthanum carbonate was used to assess the efficacy and tolerability of doses of up to 4500 mg/day elemental lanthanum in reducing phosphorus levels to within the K/DOQI recommended range.
The study was a multicentre, open-label trial. After a 1–2-week washout period, patients who had received dialysis for at least 2 months before enrolment, started lanthanum carbonate treatment at a dose of 1500 mg/day. Lanthanum carbonate tablets containing 250, 500, 750 and 1000 mg elemental lanthanum were available and the daily dose could be increased each week to achieve target phosphorus levels. After the target was reached, patients received lanthanum carbonate for the remainder of the 12-week treatment period. The primary efficacy measure in the study was pre-dialysis serum phosphorus levels. Secondary efficacy measurements included assessment of serum PTH, calcium, and calcium ′ phosphorus product. Safety was assessed by physical examination, vital signs, adverse events, and laboratory investigations.
Results from this study will determine whether the efficacy and tolerability this new formulation will lead to control of phosphorus levels to the K/DOQI recommended range, at doses of lanthanum carbonate higher than those used in previous studies.

W-PO40071

HIGH PHOSPHATE LEVEL DIRECTLY STIMULATES PARATHYROID HORMONE SECRETION BY HUMAN PARATHYROID TISSUE IN VITRO AND PHOSPHOFORMATE INTERFERES IN ONE WANG

XIAO BING WANG[1], WANGXIAOYUN[1], MAO HUIJUAN[1]
Department of Nephrology, First Affiliated Hospital, Nanjing Medicinal University, Nangfing, China[1]

High phosphate level directly stimulates parathyroid hormone secretion by human parathyroid tissue in vitro and phosphoformate interferes in one.
Objective: To evaluate the effect of high phosphate level on the secretion of parathyroid hormone (PTH) in human hyperplastic parathyroid glands (PTG) and the effect of the inhibition on PTH secreting by the phosphoformate (PFA).
Methods: Reverse transcription and polymerase chain reaction (RT-PCR) was used to identificated the expression of the sodium independed phosphate cotransporter Pit-l in parathyroid tissue of uremic patients with secondary hyperparathyroidism. Small pieces of parathyroid tissue were cultured for 6 hours, 12 hours and 18 hours and followed by aspirating the supermatant of medium respectively ($n = 6$), iPTH therein and the DNA content of the PTG tissue were measured. Some pieces of PTG were observed with microscope after 18 hours culture. There were two groups (P: 0.95 mmol/L or 3.37 mmol/L). In high-P groups, six contain PFA 0.5 mmol/L, six contain PFA 1.0 mmol/L and six do not contain PFA.
Results: Necrosis tissue was not detected with microscope. The PTH secretion was correlation with the time of culture in NP ($r = 0.721$, $p = 0.001$). The PTH contents in HP were more than ones in NP ($p < 0.05$). The PTH contents in PFA were lower than ones in HP ($p < 0.05$), ones in PFA (1.0 mmol/L) were least ($p < 0.05$). Na/P cotransporter (Pit-1) was expressed by the method of RT-PCR and Nucleic acid sequencing.
Conclusion: High phosphate levels directly affect PTH secretion in patients with advanced secondary hyperparathyroidism. PFA may prevent secretion of PTH by the Na/P cotransporter.

W-PO40072

THE INFLUENCE OF LOW CALCIUM DIALYSATE ON SERUM INTACT PARATHYROID HORMONE IN HEMODIALYSIS PATIENTS

YUQING CHEN[1], MEI WANG[1]
Renal Division and Institute of Nephrology, Peking University First Hospital, Beijing, P.R. China[1]

Background: With the widely use of calcitrol and calcium-based phosphate binder, hypercalcemia has become a new problem in hemodialysis patients. Since high calcium-phosphate product is associated with the incidence of cardiovascular disease, it is important to lower the serum calcium level in these patients. Decreasing the dialysate calcium concentration may ameliorate hypercalcemia, however the influence of low calcium dialysate on serum intact parathyroid become an attentive point.
Objective: To observe the change of intact parathyroid hormone, serum calcium and phosphate in hemodialysis patients who was treated with dialysate calcium levels of 1.25 mM (DCa1.25) for 3 months.
Method: Six patients treated with hemodialysis 3 times per week were enrolled in this study. They all received hemodialysis for 3 months with DCa1.25. During this period, the patients maintain stable for diet and medication. Before and after the using of DCa1.25 for 3 months, the serum calicium, phosphate and intact parathyroid hormone were measured in pre and post-dialysis. For a single hemodialysis the serum calicium, phosphate and intact parathyroid hormone were measured in pre-, post- and the next pre-dialysis.
Results: For a single dialysis, the serum calcium decreased in post-dialysis compared with in pre-dialysis (2.63 ± 0.16 mmol/L Vs 2.50 ± 0.23 mmol/L), and the iPTH increased significantly in post-dialysis compared with that in pre-dialysis (219.2 ± 143.3 pg/ml Vs 157.5 ± 107.1 pg/ml, p 0.05). However, the serum calcium (2.64 ± 0.15 mmol/L) and iPTH (157.7 ± 125.3 pg/ml) return to the original levels in the next pre-dialysis. When patients being dialyzed with dialysate calcium 1.25 mM for 3 months, serum iPTH increased markedly (82.5 ± 43.7 pg/ml Vs 157.5 ± 107.1 pg/ml, p 0.05).
Conclusion: When patients were dialyzed with dialysate calcium 1.25 mM for 3 months, serum iPTH increased. A long time use of dialysate calcium 1.25 mM, the iPTH should be detected regularly.

W-PO40073

THE REMNANT KIDNEY (RK) RAT AS A MODEL FOR SECONDARY HYPERPARATHYROIDISM (SHPTH)

LINE OSTE[1], V.O. VAN HOOF[1], M.E. DE BROE[1], P.C. D'HAESE[1]
University of Antwerp[1]

The RK rat has been extensively used for the assessment of bone changes due to uremia. This study aimed to better evaluate the bone histomorphometric changes in this model and assess the effect of dietary phosphorus content, duration and degree of uremia.
5/6th nephrectomy rats received a standard diet (0.67% phosphate) (NP) or a high phosphorus diet (0.93% phosphate) (HP) and were sacrificed after 6 or 12 weeks.
Serum creatinine measuerement indicated the development of moderate renal failure in both diet groups. In the HP CRF group a decrease in calciuria and a rise in phosphatemia was seen, which was more pronounced in severe CRF. Phosphaturia was significantly higher in all animals receiving the HP diet. IPTH measurement showed that only the CRF rats fed a HP diet developed sHPTH, especially those with more pronounced CRF. Serum osteocalcin was higher in the CRF animals compared to the shams and was also higher in the animals with severe CRF of the HP group. Tartrate-resistant acid phosphatase and urinary crosslinks concentrations were also higher in the CRF animals compared to the shams. Histological signs of sHPTH were only evident in the HP group: higher bone formation rate, mineral apposition rate, osteoblast perimeter and eroded perimeter. This effect was more pronounced in severe CRF. In both diet groups, the CRF animals had significantly higher osteoid accumulation compared to shams.
In conclusion, this study provides (i) reference values for different biochemical and histological parameters of bone remodelling in renal failure rats and (ii) information on the importance of the phosphorus content of the diet and the degree of renal failure in the rat remnant kidney model.

W-PO40074

EFFECTS OF MORNING VS. EVENING ADMINISTRATION OF ORAL ACTIVE VITAMIN D IN PRE-DIALYSIS PATIENTS ON SERUM CALCIUM AND OTHER PARAMETERS OF BONE METABOLISM

HENDRIK KOLLER[1], EMANUEL ZITT[1], GERT MAYER[1], ALEXANDER R. ROSENKRANZ[1]
Medical University Innsbruck[1]

Background/Objective: Active Vitamin D (1, 25-dihydroxy-cholecalciferol) supplementation is routinely administered in patients with chronic kidney disease for treatment of renal osteodystrophy. Among various effects, active Vitamin D increases intestinal Calcium absorption and may therefore lead to an increased Calcium load. This is thought to be associated with an increased cardiac morbidity and mortality in this population. We speculated whether this increase in Calcium absorption might depend on intestinal Calcium availability and the timing of the drug administration. We therefore examined the effects of morning vs. evening administration of active Vitamin D on parameters of bone metabolism.
Patients/Methods: 27 Patients with chronic kidney disease stage 3 and 4 and eligible for active Vitamin D treatment as recommended by the KDOQI-guidelines were advised to take their daily oral Vitamin D supplementation explicitly in the morning for 3 months. After this period, the patients were instructed to take their oral Vitamin D supplementation in the evening for another 3 months. During the 6 month study period, serum Ca2+, Phosphorus, PTH and Calcium-Phosphate product were measured monthly.
Results: During the 6 month study period, corrected serum Calcium levels changed from 2.33 ± 0.19 (morning Vitamin D administration) to 2.35 ± 0.19 mmol/l (evening Vitamin D administration; $p = 0.42$). There were 3 hypercalcemic episodes during the morning Vitamin D supplementation vs. none during the evening administration. Phosphorus levels changed from 1.55 ± 0.33 mmol/l to 1.64 ± 0.38 mmol/l ($p = 0.16$), PTH levels from 210 ± 166 pg/ml to 243 ± 170 pg/ml ($p = 0.31$), Calcium – Phosphate product changed from 3.60 + 0.83 to 3.84 + 0.84 $mmol^2/l^2$ ($p = 0.10$; morning vs. evening administration).
Conclusion: In our study, morning vs. evening oral active Vitamin D supplementation does not alter serum Calcium levels and other parameters of bone metabolism significantly.

W-PO40075

THE INFLUENCE OF GOLDEN SNAIL INTAKE TO SERUM PARATHYROID HORMONE LEVELS IN END-STAGE RENAL DISEASE ROUTINE HEMODIALYZED PATIENTS

MOCHAMMAD SJA'BANI[1], KUKUH SUBEKTI, SUHARDI DA, IRI KUSWADI, FREDIE IRIJANTO[1]
Department of Internal Medicine Faculty of Medicine, Gadjah Mada University, Dr. Sardjito Hospital Yogyakarta, Indonesia[1]

Background: Golden snail is a popular food in some ethnic Indonesians and end-stage renal disease (ESRD) patients who are routinely haemodialyzed especially in Dr. Sardjito Hospital, Yogyakarta. The benefits of golden snail for ESRD patients have been introduced by Sja'bani and colleagues in Renal Unit in the same hospital since 2000. That was the first time of using golden snail as a protein source for ESRD patients in Indonesia. Golden snail has low phosphate and high calcium level compared to the other meat. The high biological value and low phosphate/calcium ratio could increase dietary protein intake and prevent hyperphosphatemia. Restriction intake of dietary phosphate is able to reverse many of the abnormalities in mineral metabolism, increase calcitriol serum concentrations, while that of intact parathyroid hormone (iPTH) is diminished.
Objective: To investigate the influence of golden snail intake to the level of parathyroid hormone in ESRD patients who are routinely hemodialyzed in Dr. Sardjito Hospital, Yogyakarta.
Study Design: A cross sectional study had been done in ESRD patients who underwent maintenance haemodialysis in Renal Unit Dr Sardjito Hospital, Yogyakarta for 4–5 hours/session, twice/weekly for more than 12 weeks. Group I are patients who consumed golden snail every mealtime during ≥5 days/week while group II are patients who consumed golden snail <2 days/week, within 3 months respectively.
Result: There was significantly lower mean of serum iPTH level in Group I (205.60 ± 166.36 pg/mL) than Group II (223.90 ± 88.38 pg/mL) ($p = 0.034$; 95% CI 6.35–157.31).
Conclusion: There was a significantly lower mean of serum iPTH level in patients who consumed golden snail every mealtime during ≥5 days/week compared to patients who consumed golden snail <2 days/week, within 3 months respectively.

W-PO40076

CAN INTENSIVE DIET COUNSELLING LOWER THE PHOSPHATE BURDEN AMONG CHRONIC HEMODIALYSIS PATIENTS

HOW-CHENG LOW[1], NORELLA KONG CT[1], FAUZI AR[1], CHEE SS[1], TILAKAVATI K[1], MAT DAUD ZA[1]
National University of Malaysia[1]

Objective: To evaluate the effectiveness of intensive dietary counselling in lowering serum phosphate and iPTH levels among chronic haemodialysis patients.
Method: 48 stable chronic hemodialysis (HD) patients with elevated serum phosphate levels were randomized to either intervention or control arms and followed for 9 weeks. Both groups attended a half-day dietary phosphate workshop and were given a dietary phosphate book to take home. The intervention group also received two additional dietary counselling sessions at 3-weekly intervals.
Results: 46 patients completed the study. There were 30 males and 16 females with a median age of 50.5 ± 12.5 years (range 23–67). There were 28 Malays, 16 Chinese and 2 Indians. There were no differences beteen the groups in the serum calcium, phosphate, iPTH and [Ca × P] product over time. In the controls, the median serum phosphate reduced from 2.22 ± 0.54 to 1.96 ± 0.65 mmol/L ($p = 0.097$), iPTH reduced significantly from 57 ± 54.7 to 34.0 ± 48.4 pmol/L ($p = 0.002$), [Ca × P] product reduced from 4.81 ± 3.67 to 4.51 ± 1.62 ($p = 0.128$). In the intervention group, there was no significant reduction in the serum phosphate 2.13 ± 0.61 to 2.10 ± 0.60 mmol/L ($p = 0.077$) and iPTH levels 58.1 ± 33.4 to 49.5 ± 38.6 pmol/L ($p = 0.144$). Notwithstanding these, the [Ca × P] product improved significantly from 4.66 ± 1.21 to 4.45 ± 1.18 mmol/L ($p = 0.048$). There was an inverse association between serum phosphate and intake of CaCO3 in the intervention group but not in the control group (Control: $R = -0.238$, $p = 0.275$; Intervention: $R = -0.420$, $p = 0.046$).
Conclusion: Intensive dietary phosphate counselling conferred no added benefit over a one-off dietary workshop on blood phosphate levels in our study cohort.

W-PO40077

REVERSIBILITY OF IMPAIRED MINERALIZATION ASSOCIATED WITH LANTHANUM (LA) CARBONATE INDUCED PHOSPHATE DEPLETION (PD) IN CHRONIC RENAL FAILURE (CRF) RATS

AN RJ BERVOETS[1], LINE OSTE[1], GEERT J BEHETS[1], GEERT DAMS[1], MARC E DE BROE[1], PATRICK C DHAESE[1]
Laboratory of Nephrology-Hypertension, University of Antwerp, Belgium[1]

We have previously shown that administration of the phosphate binder La carbonate at high doses during 12 weeks induces a mineralization defect (MD) in CRF rats. In view of the unchanged number of active osteoblasts we hypothesised that the MD was secondary to the La-induced PD and was not due to a direct effect of La on bone. Here we studied the possible biological activities of La accumulated in bone after withdrawal of the compound.
In this study CRF animals ($5/6^{th}$ nephrectomy) received La carbonate (2000 mg/kg/day) for 2 or 6 weeks. Animals were then sacrificed immediately, or after a wash out (WO) period of 2 and 8 weeks and bone samples for histomorphometry and bone La measurement were taken. Controls received vehicle only.
After 2 weeks of La treatment, 75% of the animals showed signs of MD compared to 14% in CRF controls despite similar bone La levels. After a 2 weeks WO period, bone La levels remained unchanged, yet 87% showed normal bone histology for the degree of CRF. A similar evolution was noted in the animals treated for 6 weeks. Here bone La levels had increased up to 1.87 ± 0.40 μg/g (vs. 0.51 ± 0.14 μg/g in controls), and remained unchanged after a 2 or 8 weeks WO period. Bone histology, however, showed a reduction of number of animals with a MD from 64% at 6 weeks to 20% after 2 weeks WO and 28% after 8 weeks WO.
The MD may (dis) appear without any change in either the perimeter of active osteoblasts or in bone La levels. Although bone La levels remained unchanged, bone histology normalized after arrest of La administration, thereby further supporting the absence of a direct effect of La on bone and corroborating our previous data indicating a PD mechanism.

Renal Osteodystrophy – Vitamin D and Calcimimetics

W-PO40078

VITAMIN D DEFICIENCY IN HEMODIALYSIS PATIENTS WITH STAGE V CHRONIC KIDNEY DISEASE

WILLIAM FINN[1]
University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA[1]

In chronic kidney disease, there is a decrease in renal 1-a-hydroxylase activity, limiting conversion of 25-hydroxyvitamin D [25-(OH)D] to 1,25-dihydroxyvitamin D [1,25-(OH)2D]. 1,25-(OH)2D has effects on growth and differentiation of many cell types. Thus, there is a need for adequate 25-(OH)D stores.
To determine vitamin D status in hemodialysis patients, baseline levels were measured in a subset of 103 patients enrolled in a 2-year comparative study of lanthanum carbonate. Patients were^{3} 18 years of age, and had received dialysis for at least 2 months. Exclusion criteria included a screening serum calcium level <7.9 mg/dL, clinically significant concurrent illness, or serum transaminase levels more than 3′ the upper limit of normal. 25-(OH)D levels <5 pg/mL were considered severely deficient, 5–15 pg/mL mildly deficient, and 16–30 pg/mL insufficient. 1,25-(OH)2D levels <22 ng/mL were considered inadequate.
At screening, the mean (±SD) serum level of 25-(OH)D was 17.6 ± 10.9 pg/ mL (95% CI: 15.5, 19.7; range: 4.0–51.0 pg/mL). 25-(OH)D levels were severely deficient in 25% of patients. The mean (±SD) level of 1,25-(OH)2D was 9.66 ± 9.06 ng/mL (95% CI: 7.88, 11.44 ng/mL; range: 5.0–76.4 ng/mL). Overall, 88% of patients had low 1,25-(OH)2D levels.
Most hemodialysis patients require phosphate binders, most commonly calcium salts. These have been associated with an increased risk of hypercalcemia, particularly when used in combination with vitamin D analogs. Concerns about increasing serum calcium levels, an independent risk factor for mortality, may therefore lead to under-prescription of vitamin D.
Severe deficiencies in 25-(OH)D and 1,25-(OH)2D exist in a substantial percentage of hemodialysis patients. Serum levels should be monitored and appropriate replacement therapy provided.

W-PO40079

EFFECTS OF CALCITRIOL AND PARATHYROID HORMONE ON OPG AND RANKL LEVELS IN HEMODIALYSIS PATIENTS

IVICA AVBERSEK-LUZNIK[1], BREDA PECOVNIK-BALON[2], IGOR RUS[3], ANTON ADAMLJE[4], JANJA MARC[5]
Department of Clinic Biochemistry, General Hospital Jesenice, Slovenia[1], Nephrology Dept, General Hospital Maribor, Slovenia[2], Dialysis Center, General Hospital Jesenice, Slovenia[3], Dialysis Center, General Hospital Trbovlje, Slovenia[4] Faculty of Pharmacy, University of Ljubljana, Slovenia[5]

Reports from previously conducted studies indicate that parathyroid hormone (PTH) and calcitriol alter gene expression of osteoprotegerin (OPG) and receptor activator of nuclear k B ligand (RANKL). PTH inhibits OPG and stimulates RANKL synthesis in primary cell cultures, while calcitriol transiently stimulates the synthesis of both cytokines. We examined the effects of calcitriol administration on serum OPG and RANKL levels in hemodialysis (HD) patients. 0.25 to 0.50 mg doses of calcitriol were administered to 26 patients following dialysis session two times weekly.
Blood samples were taken prior to regular hemodialysis procedure from 26 calcitriol treated and 80 HD patients without such treatment. In addition to OPG, RANKL and PTH levels, established bone markers: bone alkaline phosphatase (bALP), osteocalcin (OC), tartarate resistant acid phosphatase 5b (TRAP 5b) and cross Laps (CTx) were determined in serum samples of both groups. All markers were determined using specific enzyme immunoassay methods.
Serum levels of PTH were slightly higher in calcitriol treated than in nontreated group ($p = 0.088$). Further we found significantly higher RANKL levels ($p = 0.001$). Serum levels of OC approached the limit of significance ($p = 0.072$), while there was no considerable difference in levels of OPG ($p = 0.355$), bALP ($p = 0.147$), CTx ($p = 0.350$) and TRAP 5b ($p = 0.697$) between both groups. It is worth mentioning that there was a tendency to higher levels of OPG and measured bone markers in the group of patients treated with calcitriol.
Considering our results we concluded that significantly increased RANKL levels in calcitriol treated HD patients are due to stimulating effects of calcitriol and/or PTH on RANKL expression in osteoblasts precursor cells. Consequently larger pool of mature osteoblasts might be responsible for a relatively increased OPG synthesis. We speculate that additional as yet unknown mechanisms can also contribute to the regulation of RANKL and OPG synthesis in HD patients.

W-PO40080

EVALUATION OF 25(OH) VITAMIN D SERUM LEVELS IN UREMIC PATIENTS IN HEMODIALYSIS (HD)

ARMANDO LUIS NEGRI[1], GUILLERMO ROSA DIEZ[2], ELISA DEL VALLE[1], CRISTINA AGUIRRE[1], JULIANA FASSI[2], LUISA PLANTALECH[2], LUIS ALGRANATI[2], ERICH FRADINGER[1], JOSE RUBEN ZANCHETTA[1]
Instituto de Investigaciones Metabólicas[1], Hospital Italiano[2]

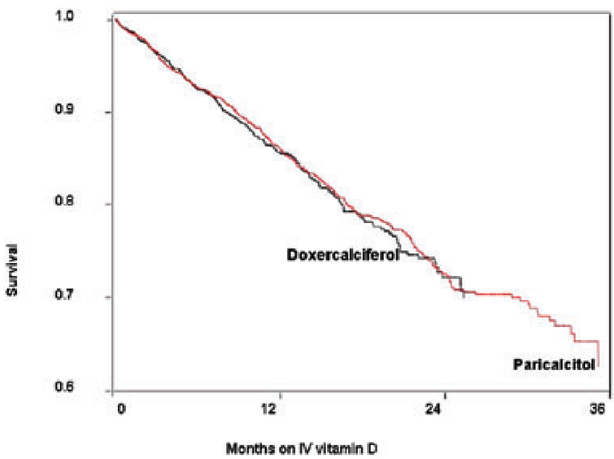
Little is known about the magnitude of vitamin D deficiency in patients with stage 5 Chronic Kidney Disease (CKD). Thus we evaluated serum 25(OH) vitamin D levels in two groups of stable patients in chronic HD (45 months) of both sexes. Group S ($n = 94$; mean age 57.2 ± 18 years) was studied during summer and group W ($n = 84$; mean age 58.9 ± 16.6 years) at the end of winter. 25(OH) vitamin D serum levels were measured by RIA (Diasorin, Stillwater, Minesotta, USA; Interassay CV <15%). Intact PTH was measured by an automated method (Elecsys). We also determined serum albumin, creatinine, PCR and BMI as nutritional parameters and functional capacity (FC) according to Karnofsky index. We considered adequate vitamin D levels those >30 ng/ml; vitamin D insufficiency 30 to 10 ng/ml and vitamin D deficiency <10 ng/ml. We found vitamin D insufficiency in 52% and vitamin D deficiency in 3.2% of patients in group S and 64.2% and 11.9% in group W. There was a positive correlation of 25(OH) vitamin D serum levels with serum albumin (Group S $r = 0.28$; group W $r = 0.22$) and with creatinine ($r = 0.41$ and $r = 0.38$ respectively). Patients with FC 1–2 (group S 62% and group W 83.3%) had a significantly higher 25 OH D serum levels compared with patients FC 3–4 (group S 38% and group W 16.6%) ($P = 0.03$). These data indicate that vitamin D insufficiency/deficiency is highly prevalent in patients stage 5 CKD on dialysis (56.4% in summer and 76.1% at the end of winter)) and it seems to be related to poorer nutrition and worse functional class.

W-PO40081

SURVIVAL DOES NOT DIFFER AMONG HEMODIALYSIS PATIENTS RECEIVING DOXERCALCIFEROL OR PARICALCITOL

FRANCESCA TENTORI[1], CURTIS HUNT[1], CHRISTINE STIDLEY[1], DOUG LINDSEY[1], PHILIP ZAGER[1]
Dialysis Clinic Inc. (DCI), University of New Mexico[1]

IV vitamin D analogs are standard therapy for secondary hyperparathyroidism among hemodialysis (HD) patients. Improved survival has been described for patients receiving paricalcitol versus calcitriol. We postulated that mortality risk is similar among patients receiving doxercalciferol and those receiving paricalcitol.
We analyzed data from incident HD patients in Dialysis Clinic Inc. (DCI) facilities between 2001 and 2003. 1,723 patients were started on doxercalciferol and 1,667 or paricalcitol. Hazard ratios (HR) for all-cause mortality for patients receiving paricalcitol versus doxercalciferol were calculated using Cox proportional models adjusted for demographics (age, gender, race, cause of ESRD, vintage, year vitamin D analog was started) and baseline labs (creatinine, Kt/V, HCT, ferritin, albumin, PTH, calcium and phosphorus).
Kaplan-Meier survival curves for patients receiving doxercalciferol and paricalcitol are shown:



No difference in mortality risk was observed between patients receiving doxercalciferol compared to those on paricalcitol (HR: 1.0; 95% Confidence Interval: 0.8–1.2). The narrow confidence interval indicates that the HR is estimated with high precision.
Since no difference in survival was demonstrated, these results indicate that either paricalcitol or doxercalciferol can safely be prescribed.

W-PO40082

DECREASED ODDS OF HOSPITALIZATION AMONG HEMODIALYSIS PATIENTS RECEIVING DOXERCALCIFEROL VERSUS CALCITRIOL

PHILIP ZAGER[1], CURTIS HUNT[1], CHRISTINE STIDLEY[1], DOUG LINDSEY[1], FRANCESCA TENTORI[1]
Dialysis Clinic Inc. (DCI), University of New Mexico[1]

IV vitamin D analogs are standard therapy for secondary hyperparathyroidism among hemodialysis (HD) patients. Lower hospitalization rates have been reported among patients receiving paricalcitol compared to calcitriol. We postulated that hospitalizations would also be less frequent among patients receiving doxercalciferol versus calcitriol.
We analyzed data from incident HD patients who were treated at Dialysis Clinic Inc. (DCI) facilities between 2001 and 2003. During the study period, 1,083 patients were started on calcitriol, 1,624 on paricalcitol and 1,667 on doxercalciferol. The odds for hospitalization in repeated 14-day intervals following the initiation of IV vitamin D were compared by type of vitamin D analog using logistic regression with generalized estimating equations. Models were adjusted for demographics (age, gender, race, cause of ESRD, vintage, year vitamin D analog was started), baseline labs (creatinine, Kt/V, HCT, ferritin, albumin, PTH, calcium and phosphorus) and number of hospitalizations prior to the start of the interval.
The odds for hospitalization were significantly lower among patients receiving doxercalciferol compared to those on calcitriol (odds ratio: 0.90; 95% Confidence Interval: [0.82–0.99]). No significant difference in the odds for hospitalization was observed for paricalcitol versus doxercalciferol (1.03[0.95–1.12]).
In summary, we demonstrated that the odds for hospitalization were significantly lower among patients receiving doxercalciferol compared to those on calcitriol. Considering the high economical expense related to hospitalizations, the use of doxercalciferol may decrease the global cost of treating HD patients.

W-PO40083

THE CALCIMIMETIC R568 LOWERS BLOOD PRESSURE BUT NOT TOTAL BODY SODIUM CONTENT IN RATS

CLAUS PETER SCHMITT[1], NAKAGAWA K[1], HADTSTEIN C[1], ODENWALD T[1], ROESCH F[2], SCHAEFER F[1], RITZ E[3]
Pediatric Nephrology, University of Heidelberg, INF 153, 69120 Heidelberg, Germany[1], Institut für Kernchemie, F.-Strassmann-Weg 2, 55128 Mainz, Germany[2], Nephrology, Heidelberg[3]

Gain-of-function mutations in the Calcium receptor reduce NaCl reabsorption in the thick ascending limb, resulting in a negative net sodium balance. Calcimimetics have been shown to lower blood pressure (BP) in SNX rats. One possible explanation is that calcimimetics reduce total body sodium (TBS) and thus BP.
We administered R568 (20 mg/kg*d) or solvent to male SD rats by osmotic minipumps for 14d. The animals ($n = 9$ per group) received normal or high phosphate diets to stimulate PTH secretion (NPD: 0.7%, HPD: 1.65% phosphate) and similar dietary sodium. TBS was determined by neutron activation analysis after sacrifice and removal of the gastrointestinal tract. BP was measured by tail cuff-plethysmography after 7 days. Telemetric BP monitoring was performed in additional animals on NPD receiving R568 or solvent ($n = 5$ and 4).
Surprisingly, TBS was not decreased but even significantly increased in R568 treated rats on NPD compared to solvent controls (37.1 ± 4.0 vs. 32.5 ± 1.4 mmol/kg, $p = 0.01$). In HPD rats TBS did not differ between the two groups (38.9 ± 4.0 vs. 40.7 ± 3.8 mmol/kg, p = ns). In R568 treated rats on NPD systolic BP was significantly lower after 7d compared to controls (126 ± 8 vs. 143 ± 2 mmHg, $p < 0.005$). The BP reduction was similar on HPD and NPD.
Systolic/diastolic BP measured telemetrically was reduced by $-13 \pm 12/-8 \pm 3$ mmHg after 14d R568 treatment, but only $-2 \pm 2/-2 \pm 4$ mmHg in solvent treated animals ($p < 0.001$). Blood ionized calcium decreased in R568 treated rats (NPD: 1.11 ± 0.08; HPD: 1.05 ± 0.04 mmol/l) but not in solvent controls (NPD: 1.38 ± 0.07, HPD: 1.33 ± 0.05 mmol/l, day 7, $p < 0.001/<0.001$), sodium, potassium and pH remained unchanged.
The calcimimetic R568 reduces BP in rats without inducing sodium depletion. The increase in TBS in rats fed a NPD may reflect compensatory sodium retention in response to lowered BP. The finding points to beneficial effects of calcimimetics beyond control of calcium and phosphate.

W-PO40084

EFFECTS OF CINACALCET ON BONE MINERAL DENSITY IN PATIENTS WITH SECONDARY HYPERPARATHYROIDISM

YEONG-HAU HOWARD LIEN[1], ARNOLD L. SILVA[1], DAVID WHITTMAN[1]
University of Arizona[1]

Background: Cinacalcet, a calcimimetic agent, is effective in treating both primary and secondary hyperparathyroidism. Because hyperparathyroidism induces mineralized bone loss, we investigated the effects of cinacalcet treatment on bone mineral density in patients with secondary hyperparathyroidism due to chronic kidney disease.
Methods: Ten patients, who were receiving hemodialysis, and 4 patients, who had stage 4 chronic kidney disease, participated and completed the multi-center, randomized, double-blind, placebo-controlled trials evaluating the safety and efficacy of cinacalcet for treating secondary hyperparathyroidism. The efficacy of cinacalcet was assessed by plasma intact parathyroid hormone (iPTH) levels. A dual energy X-ray absorptiometry (DEXA) was performed to measure bone mineral density (BMD) of proximal femurs and lumbar spine (L2-L4) before and after treatment.

Results: Cinacalcet reduced iPTH from 912 ± 296 to 515 ± 359 pg/ml in hemodialysis patients and from 210 ± 46 to 56 ± 51 pg/ml in pre-dialysis patients (Means ± SD; both $P < 0.05$). When data from hemodialysis and pre-dialysis patients were pooled for analysis, cinacalcet treatment increased proximal femur BMD from 0.945 ± 0.169 to 0.961 ± 0.174 g/cm^2 ($P < 0.05$), but did not affect lumbar spine BMD. There was a correlation between the change in femur BMD and the change in iPTH during the study period ($R^2 = 0.39$, $p < 0.05$).
Conclusions: Secondary hyperparathyroidism is associated with progressive bone loss. Suppression of plasma iPTH with cinacalcet appears to reverse bone loss in proximal femurs, but does not affect BMD of the lumbar spine. A larger study is warranted to confirm that cinacalcet has a beneficial effect on the skeletal system in patients with secondary hyperparathyroidism.

W-PO40087

THE EFFECT OF 25 HYDROXYLATED VITAMIN D SUPPLEMENTATION IN HAEMODIALYSIS PATIENTS-A CONTROLLED STUDY

STEPHEN BENEDICT WALSH[1], TÜRK T[2], CHAMPENOIS I[2], UNWIN R[3], CUNNINGHAM J[3], JAEGER PH[2]
St Peter's Renal Unit, University College Hospital LONDON, United Kingdom[1], Renal Department University of Nice FRANCE[2], Renal Unit University College Hospital London UK[3]

Background: Serum 25-hydroxyvitamin D (25(OH)D) levels are an index of vitamin D deficiency; a level <30 ng/mL is an accepted criterion for deficiency. In patients with chronic renal failure (CRF), reduced 25(OH)D levels predispose to secondary hyperparathyroidism. In a controlled study we monitored the effect of oral 25(OH)D supplementation on serum calcium (Ca), phosphate (Pi) and parathyroid hormone (PTH) levels in a cohort of CRF (haemodialysis) patients over 6 months.
Methods: 47 patients had baseline serum 25(OH)D levels <30 ng/mL (group A) and 34 patients (group B) had levels >30 ng/mL. Usual treatment, including the use of activated 1-hydroxylated vitamin D metabolites was unchanged and the only difference was supervised oral administration of 25(OH)D (35 mg) to group A ×3 per week.
Results: Except for PTH (log-normal), data were normally distributed and analysed by paired t-test, comparing baseline and 6 months. Serum 25(OH)D levels increased in group A (15.6 ± 1.1 to 31.1 ± 2.7 ng/mL; $P < 0.01$) and decreased in group B (63.5 ± 6.8 to 24.7 ± 3.5 ng/mL; $P < 0.01$). Mean Pi concentration increased moderately, but significantly, in treated patients (1.77 ± 0.08 to 1.97 ± 0.09 mM, $P < 0.05$), whereas in group B no significant change occurred. There were no significant changes in serum PTH concentration in either group (mean of 190.1 to 184.5 and 133.9 to 150.6 pg/ml in groups A and B respectively) or in Ca. Ca × Pi phosphate product in group A did increase significantly (4.21 ± 0.20 to 4.66 ± 0.24 mM2; $P < 0.02$), though it did not in group B.
Conclusion: Modest 25(OH)D supplementation, sufficient to correct an underlying vitamin D deficiency significantly increased Pi concentration and the Ca × Pi product, without materially affecting Ca or PTH. These results suggest an effect of 25(OH)D supplementation on intestinal Pi absorption, a potentially unfavorable effect of correcting vitamin D deficiency according to current guidelines.

Renal Osteodystrophy – Diagnosis and Treatment

W-PO40088

SUCCESSFUL TREATMENT OF VERY SEVERE OSTITIS FIBROSA WITH DIRECT MAXACALCITOL INJECTION INTO PARATHYROID GLAND

KAZUHIRO SHIIZAKI[1], SHIGEO NEGI[2], TOSHIFUMI SAKAGUCHI[2], IKUJI HATAMURA[2], KEN KUNIMOTO[2], MASANORI OKAMOTO[2], IKUO IMAZEKI[2], TADAO AKIZAWA[2]
Wakayama Medical University[1], Chugai Pharmaceutical Co. Ltd.[2]

Direct injection of maxacalcitol (OCT) into parathyroid gland (PTG) ameliorates several important etiologic factors of resistance to the medical treatments for secondary hyperparathyroidism (SHPT); the up-regulation of vitamin D receptor and Ca-sensing receptor in parathyroid cell (PTC) and the regression of hyperplasia by the induction of apoptosis in PTC. This study evaluated the effects of direct OCT injection into PTG on bone histomorphology in very severe SHPT.
5/6 nephrectomized male Sprague-Dawley rats were fed high-phosphate diet (1.2% P, 0.4% Ca) for eight weeks. These rats were divided into four treatment groups; 1) Baseline, 2) No treatment for four weeks, 3) Direct OCT single injection into bilateral PTG and following OCT intravenous administration (2.5 μg/kg) (three times per week for four weeks) (IV-OCT), and 4) Direct vehicle single injection into bilateral PTG and IV-OCT. The effects of these treatments on time course change in serum intact-PTH level and bone histomorphology were investigated.
In the group of direct OCT injection with IV-OCT, serum intact-PTH level was significantly decreased (27% of the level before treatment) and this suppression was maintained for four weeks by the following IV-OCT. In bone histomorphological examination, the lamellar structure, the significant decrease in fibrosis volume, the regular labeling of calcein in cancellous bone and the significant decrease in bone width and porosity area in cortical bone were observed. However, these findings were never observed in other groups.
These results suggested that the bone disease caused by very severe SHPT was successfully reversed by the sustained PTH suppression. The cellular effects on PTC of direct OCT injection into PTG may make it possible to avoid the surgical removal of PTG in severe SHPT.

W-PO40089

BONE PARAMETERS AND BONE MINERAL DENSITY IN HEMODIALYSIS PATIENTS AT START AND AFTER 1 YEAR OF DIALYSIS THERAPY COMPARED TO OSTEOPOROSIS PATIENTS

ROMAN FIEDLER[1], MARGIT MALL[1], CLEMENS WAND[1], DOREEN THIEMICKE[1], BERND OSTEN[1]
Dept of Nephrology, Martin-Luther-University Halle-Wittenberg, Germany[1]

Recently was published that osteoporosis in hemodialysis patients is an independent risk factor of cardiovascular mortality. However it is unknown how many hemodialysis patients suffer from significant osteoporosis (T-Score < –2.5 SD). The aim of our study was to compare bone parameters and osteodensitometry in hemodialysis versus osteoporosis patients.
We investigated 4 groups of age-matched patients, group A = 27 hemodialysis patients at start of chronic dialysis therapy (age: 62 ± 17 years), group B = 17 hemodialysis patients after 1 year of chronic dialysis treatment (age: 62 ± 16 years), group C = 19 osteoporosis patients (age: 66 ± 11 years), and group D = 18 osteoporosis patients (age: 64 ± 10 years). Levels of intact parathyroid hormone (PTH), 25-OH-vitamin D (25-D3), and bone-specific alkaline phosphatase as well as bone mineral density (BMD) at lumbar spine and/or femoral neck were determined.
PTH concentrations in groups A (19.7 ± 19.4 pmol/l, $p < 0.01$) and B (14.6 ± 21 pmol/l, $p < 0.05$) were significant increased compared to groups C (4.2 ± 2 pmol/l) and D (3.4 ± 1.1 pmol/l), but 25-D3 levels were declined in groups A (30 ± 18 nmol/l, $p < 0.05$) and B (34 ± 22 nmol/l, n.s.) versus C (46 ± 32 nmol/l) and D (55 ± 48 nmol/l). Despite important secondary hyperparathyroidism and vitamin D deficiency BMD was significant higher in hemodialysis groups A (T-Score: -0.56 ± 1.52) and B (T-Score: -0.62 ± 1.62) than in osteoporosis groups C (T-Score: -2.3 ± 0.86) and D (T-Score: -4.03 ± 1.11) at lumbar spine ($p < 0.001$) and at femoral neck ($p < 0.05$). Rates of osteoporosis were 33% at start and 29% after 1 year of hemodialysis therapy.
In dialysis patients osteoporosis should not be underestimated. Examined bone markers don't play a role for the diagnosis of osteoporosis, but BMD assessment is recommendable in hemodialysis patients suspicious on osteoporosis.

W-PO40090

REVERSAL OF 1–84 PTH VALUE AGAINST IPTH VALUE IN A SEVERE SECONDARY HYPERPARATHYROIDISM PATIENT

TOSHIO ARAKAWA[1], NAOYA IGAKI[1], MAKOTO SAKAI[2], TAKEO GOTO[2], MASAFUMI FUKAGAWA[3]
Arakawa Renal Clinic[1], Takasago Municipal Hospital[2], Kobe University School of Medicine Division of Nephrology[3]

Background: Recently a new method of measuring complete parathyroid hormone (1–84PTH) has been established, and the value of circulating 1–84PTH is found to be around 40% of the value measured by the intact PTH IRMA (iPTH). However, some peculiar cases were reported to show higher values of 1–84PTH than those of iPTH.

In the present study, we analyzed the sera and a parathyroid gland in a dialysis patient with higher values of 1–84PTH than those of iPTH

Method: In the sera before and after parathyroidectomy, 1–84PTH and iPTH were measured, and a removed parathyroid gland was investigated pathologically.

Results: Before parathyroidectomy, 1–84PTH values were higher than iPTH values, but after operation, this reversal phenomenon disappeared. The histological section of the removed parathyroid gland revealed severe nodular hyperplasia without malignancy (single nodule).

Conclusions: Novel form of PTH which was not recognized by conventional iPTH assay (iPTH IRMA) was discovered in secreted sera from a parathyroid gland of a dialysis patient with severe hyperparathyroidism. A few cases with parathyroid carcinoma were also reported to demonstrate this novel form of PTH (Rubin et al.) and to show higher values of 1–84PTH than those of iPTH, namely reversal phenomenon, but this is the first time to report such a phenomenon even in a dialysis patient with severe hyperparathyroidism. Thus, it is of clinical importance to measure both 1–84PTH and iPTH values in order to detect specified pathological status like parathyroid carcinoma or advanced form of parathyroid hyperplasia recognized in dialysis patients.

W-PO40091

APPROPRIATE SERUM LEVEL OF ALKALINE PHOSPHATASE FOR TREATMENT WITH CALCITROL IN HEMODIALYSIS PATIENTS

NADER NOURI[1], HOSHANG SANADGOL[2], FARZANH NAJAFIE[1], MOHAMAD RAHIMIAN[1]

Department of Nephrology, Sadoky Medical University, Yazd, Iran[1], Department of nephrology, Zahedan Medical University, Zahedan, Iran[2]

Serum parathyroid hormone (PTH) is one of the most important test for treatment of hemodialysis patient with calcitriol and this drug should be started when the PTH ≥200 pg/ml. However, the appropriate level of serum alkaline phosphatase (ALP) for starting of calcittriol is unclear. The aim of this study was to determine a serum ALP as cutoff point for treatment of secondary hyperparathyroidism with calcitriol.

This was a cross sectional study. The serum level of PTH and ALP was measured in 75 chronic hemodialysis patients.

The correlation between PTH and ALP in diagnosis for treatment with calcitrol was significant (Kappa test p-value = 0.000). In determination of diagnosis value of ALP in comparison with PTH for treatment with calcitrol, sensitivity, specificity, positive predictive value, negative predictive value and accuracy were 88.9%, 93.9%, 66.7%, 98.4% and 93.3%, respectively (Table 1). The cutoff point of ALP for treatment with calcitrol was determined by receiver operator characteristic (ROC) curve and it was 300 IU/l (normal 100–290 IU/l) (Figure 1).

These findings suggest that serum ALP is a good test for treatment of secondary hyperparathyroidism. The results also indicate that calcitrol administration should be started with ALP >300 IU/ml.

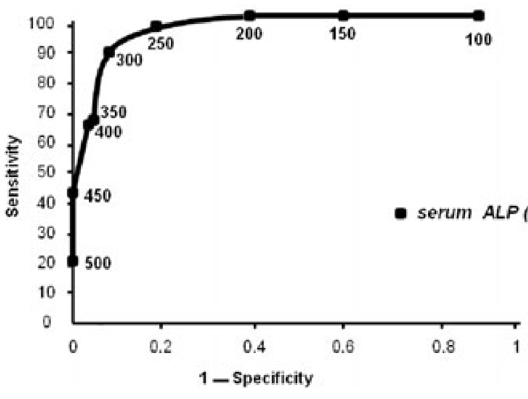


Fig. 1 Receiver operator characteristic (ROC) curve for serum ALP in the diagnosis of secondary hyperparathyroidisim that need to treatment with calcitrol (ALP of 300 IU/I is more sensitivity and specificity)

Table 1 Determining sensitivity and specificity of serum ALP for calcitriol therapy

	PTH < 200	PTH ≥ 200
patients with ALP < 300	62 (82.7%)	1 (1.3%)
patients with ALP ≥ 300	4 (5.3%)	8 (10.7%)

Kappa test, p-value = 0.000 sensitivity: 88.9%, specificity: 93.9%

W-PO40092

RANDOMIZED CONTROLLED TRIAL ON THE USE OF PAMIDRONATE PRIOR TO TOTAL PARATHYROIDECTOMY FOR HIGH RISK TERTIARY HYPERPARATHYROIDISM PATIENTS

KUAN WENG GO[1], WONG HIN SENG[1], HISHAM AN[2], ZAKI MORAD[1]

Nephrology Department, Hospital Kuala Lumpur, Malaysia[1], Endocrine Surgery Department, Hospital PutraJaya, Malaysia[2]

Objectives: To evaluate the role of pamidronate prior to total parathyroidectomy in patient with high risk tertiary hyperparathyroidism.

Patients and methods: All patients who were listed for high risk parathyroidectomy at the Department of Nephrology, Hospital Kuala Lumpur from 1st July 2004 to 31st December 2004 were randomized to two groups, group 1 receives two doses of intravenous pamidronate 30 mg at one month and one week prior to surgery and group 2 were not given any intravenous pamidronate. Post operatively; both groups were then subjected to the usual four hourly calcium monitoring and intravenous calcium infusion. All calcium results and total calcium infusion rate were calculated.

Results: A total of 14 patients were randomized, 8 patients to group 1 (pamidronate) and 6 patients to group 2 (no pamidronate). There were no significant differences between the baseline demographic factors; age, duration of follow-up and baseline calcium, phosphate, intact parathyroid hormone (iPTH) and alkaline phosphatase level (ALP); $p > 0.05$. All patients in both groups had autotransplantation of the parathyroid gland to the sternocleidomastoid muscle. The lowest serum calcium (2.14 ± 0.14 vs 2.20 ± 0.19 mmol/L, $p > 0.05$) and mean calcium (2.57 ± 0.21 vs 2.61 ± 0.06 mmol/L, $p > 0.05$) were similar. Duration of calcium infusion (69 ± 25 vs 88 ± 40 hours, $p > 0.05$), total calcium infusion required (333 ± 249 vs 346 ± 185 g, $p > 0.05$) and duration of stay in the hospital post parathyroidectomy (6.2 ± 1.2 vs 7.2 ± 1.9 days, $p > 0.05$) were similar in both groups. No adverse symptoms of hypocalcaemia were noted in both groups of patients.

Conclusion: There were no differences in the immediate outcome of high risk patients post parathyroidectomy whether they receive pamidronate or not prior to the surgery. More patients have to be randomized to confirm the above findings.

W-PO40093

OUTCOME OF PATIENT UNDERGOING TOTAL PARATHYROIDECTOMY FOR TERTIARY HYPERPARATHYROIDISM WITH AUTOTRANSPLANTATION COMPARED WITH PATIENTS WITHOUT AUTOTRANSPLANTATION

KUAN WENG GO[1], WONG HIN SENG[1], HISHAM AN[2], ZAKI MORAD[1]

Nephrology Department, Hospital Kuala Lumpur, Malaysia[1], Endocrine Surgery Department, Hospital PutraJaya, Malaysia[2]

Objectives: To evaluate the effect of auto and non auto transplantation in total parathyroidectomy for treatment of tertiary hyperparathyroidism.

Patients and methods: All patients who were listed for parathyroidectomy at the Department of Nephrology, Hospital Kuala Lumpur and Department of Endocrine Surgery, Hospital PutraJaya from 1st July 2004 to 31st December 2004 were prospectively collected. Decision for auto transplantation was collectively made by the managing surgeon and nephrologist based on patients age and option for future renal transplant. Post operatively all patient were subjected to calcium monitoring and intravenous calcium infusion in attempt to maintain serum calcium corrected with albumin between 2.10–2.37 mmol/L.

Results: Of a total of 26 patients undergoing parathyroidectomy, 19 (73.1) patients had autotransplantation compared to 7 (26.9%) patients without autotransplantation. Except for age (39.5 ± 8.8 vs 50.3 ± 5.4 years; $p = 0.002$), there were no significant differences between the baseline demographic factors in term gender, race and baseline investigation; serum calcium, phosphate, serum intact parathyroid hormone (iPTH) and alkaline phosphatase level (ALP).

The lowest serum calcium (2.19 ± 0.18 vs 2.06 ± 0.22 mmol/L; p > 0.05) and mean calcium (2.60 ± 0.18 vs 2.74 ± 0.40 mmol/L; p > 0.05) for both groups were similar. Duration of calcium infusion and duration of stay in the hospital were similar in both groups (82 ± 37 vs 118 ± 39 hours; 6.6 ± 1.5 vs 8.6 ± 3.3 days, p > 0.05). Autotransplanted group required higher total calcium infusion (286 ± 207 vs 77 ± 97 g, p = 0.019).
No adverse symptoms of hypocalcaemia were noted in both groups of patients (p > 0.05). Patient's dose requirement for vitamin D and calcium supplements at discharge were similar in both groups.
Conclusion: There were no differences in the immediate outcome of patients post parathyroidectomy whether they were subjected to autotransplantation or non-autotransplantion.

W-PO40094

CORRELATION BETWEEN DXL AND DXA IN ASSESSMENT OF BONE STRUCTURE IN HEMODIALYSED PATIENTS

MITRA MAHDAVI-MAZDEH[1], AKBAR SOLTANI[2], BAGHER LARIJANI[2], ZOHREH HAMIDI[2], SIMA MAZIAR[1], MOHAMADREZA KHATAMI[1]
Faculty of Medicine-TUMS[1], Endocrinology & Metabolism Research Center_TUMS[2]

Introduction: Many patients under hemodialysis, have osteopenia or even osteoporosis by the definition of the World Health Organization based on bone mineral density (BMD). Dual-energy X-ray absorptiometry (DXA) of femur and spine, the standard method for diagnosis of osteoporosis, needs nonportable devices that are not available everywhere. DXL (DXA of calcaneus) is a mobile and less expensive method for assessing the bone. There is little data about this method's correlation with DXA in patients undergoing hemodialysis. The present study assessed the value of DXL in detecting changes in bone structure in hemodialysed Patients compared with DXA.
Patients & Methods: In a cross-sectional analysis, 32 patients (21 men) with a mean age of 48.31+–15.48 years and mean dialysis time of 39.53+–37.11 months (4–144), were studied. A GE-Lunar device (DPX-MD) used for DXA of the hip (neck and total) and spine. DXA of the calcaneus measured using a Demeteck device.
Results: Osteoporosis has been found in 46.9% of patients in any of the total of femur or neck of femur or L2-L4 regions (29% in Neck, 48.4% in Total, 34.4% in L2-L4 regions). Using DXL, osteoporosis diagnosed in 19.4% of patients. Using ROC curve, sensitivities of T-score ≥–2.5 of calcaneus for diagnosing of osteoporosis in neck and total of hip and L2-L4 regions were respectively 54.5% and 77.8% and 50% and specificities were respectively 90.5% and 95.5% and 84%. Area under curve for neck, total and spine regions were 0.820 (P value = 0.003), 0.955 (P value = 0.000), 0.720 (P value = 0.099), respectively. So there was not a significant relation between two these methods in spinal region.
DXL (DXA of calcaneus) can be recommended for screening of osteoporosis among hemodialysed patients. Those suspected of osteoporosis, should be examined by additional DXA measurement for establishment of diagnosis before initiation of therapy

W-PO40095

CORRELATION BETWEEN QUS OF PHALANX AND DXA IN ASSESSMENT OF BONE STRUCTURE IN HEMODIALYSED PATIENTS

SIMA MAZIAR[1], MOHAMAD PAJOOHI[2], BAGHER LARIJANI[2], ZOHREH HAMIDI[2], MITRA MAHDAVI_MAZDEH[1], MAHBOOB LESSAN_PEZESHKI[1], SEPIDEH SEIFI[1]
Faculty of Medicine_TUMS[1], Endocrinology & Metabolism Research Center_TUMS[2]

Introduction: Many patients under hemodialysis, have osteopenia or even osteoporosis by the definition of the World Health Organization based on bone mineral density (BMD). Dual-energy X-ray absorptiometry (DXA), the standard method to assess BMD, is not always available. Quantitative ultrasound (QUS) of phalanx is an inexpensive, mobile, and radiation-free diagnostic alternative. Few data there is about correlation of this method with DXA in patients undergoing hemodialysis. The present study assessed the value of QUS in detecting changes in bone structure in hemodialysed patients compared with DXA.
Patients & Methods: In a cross-sectional analysis, 64 patients (37 men) with a mean age of 51.33+–15.20 years and mean dialysis time of 49.45+–45.62 months (2–180), were studied. Fifteen of women were menopause. BMD of the hip (neck and total) and spine was measured using a GE-Lunar DXA device (DPX-MD). QUS of phalanx was done in all of them using a DBM-Sonic 1200 device. This device measures amplitude dependent speed of sound (Ad-SOS).
Results: DXA measurements established the diagnoses of osteoporosis in 31.3% in any of the total of femur or neck of femur or L2-L4 regions (25% in Neck, 18.8% in Total, 7.9% in L2-L4 regions). Using QUS of phalanx, osteoporosis diagnosed in 28.1% of patients. Sensitivities of T-score ≥–2.5 of phalanx for diagnosing of osteoporosis in neck and total of hip and L2-L4 regions were respectively 37.5% and 50% and 80% and specificities were respectively 75% and 77% and 76%. 95% CI (confidence interval) of area under of curve for diagnosis of osteoporosis in neck region contained diagonal line (p values = 0.22), so there was not a significant relation between two these methods in neck region.
QUS of phalanx can be recommended for screening osteoporosis among hemodialysed patients. Those suspected of osteoporosis, should be examined by additional DXA measurement for establishment of diagnosis.

W-PO40096

INFLUENCE OF BONE BIOCHEMICAL MARKERS IN DIALYSIS PATIENT SURVIVAL

SHAHNAZ SHAH FIRDAUS KHAN[1], ROZINA GHAZALI[2], FAN KIN SING[3]
Hospital Tengku Ampuan Rahimah, Klang, Selangor, Malaysia[1], Hospital Pulau Pinang[2], Gleneagles Medical Centre, Kuala Lumpur[3]

Objective: To study the influence of bone biochemical marker [corrected serum calcium (Ca), phosphate (PO4), calcium phosphate product (CaXPO4) and intact parathyroid hormone (iPTH)] in dialysis patient survival.
Methods: All dialysis patients under the follow up of participating centres involved with National Renal Registry (NRR) starting from 1st January1993 to 31st December 2002 were included. Patients' data were obtained from NRR.
Results: There were 7025 patients' biochemical data available for analysis. The mean Ca was 2.3 – 2.4 mmol/l and mean PO4 was 1.8–1.9 mmol/l over this 10 year period. The mean CaXPO4 product was between 4.3 to 4.5 $mmol^2/l^2$. The mean iPTH ranged from 118 – 420 ng/L. There was a decreasing trend of iPTH from 1994 to 1998 after which the levels plateau. Patient survival was analyzed against Ca, PO4, CaXPO4 and iPTH levels. The survival was adjusted for age, gender, diagnosis of primary disease, duration of renal replacement therapy and modality. Hazard ratio was 1.56 and 1.76 for Ca of <2.2 and ≥2.6 mmol/l respectively. PO4 of <1.43 and ≥2.6 mmol/l was associated with hazard ratio of 1.43 and 1.81 respectively. Hazard ratio was 1.41 and 1.23 for CaXPO4 product of <3.5 and ≥5.5 $mmol^2/l^2$. iPTH of <100 and ≥250 ng/L were associated with a hazard ratio of 1.59 and 1.19 respectively.
Conclusion: The adjusted survival curves showed a U shaped distribution for all parameters (Ca, PO4, CaXPO4, and iPTH) with extremes of each parameter were associated with poorer survival.

W-PO40097

A HIGH BODY MASS INDEX (BMI) IS ASSOCIATED WITH A MORE PRECOCIOUS AND A MORE AGGRESSIVE HISTOLOGICAL PATTERN OF SECONDARY HYPERPARATHYROIDISM (SHPTH)

CARLO BASILE[1]
Department of Nephrology, Miulli General Hospital, Acquaviva delle Fonti, Italy[1]

Hyperphosphatemia represents one of the most important factors in the development of sHPTH. We hypothesized that a higher BMI may be the expression of a larger body burden of phosphate (P), thus influencing the severity of sHPTH. Aim of this prospective study was to analyze the histological patterns of parathyroid glands and the short-time behavior of serum calcium (Ca) and P after first parathyroidectomy (PTx) in 36 patients (dialysis vintage 102 ± 63 months, BMI 23.7 ± 3.8). Nodular hyperplasia was considered a marker of histological severity and a score was attributed accordingly. Serum Ca and P were measured pre-PTx and in the 5 days post-PTx; serum PTH levels were measured pre-PTx and 3 days post-PTx. Post-PTx serum Ca, P and PTH levels were significantly lower than pre-PTx. A significant direct correlation was found between BMI and pre-PTx serum P (r 0.41; p < 0.05). Patients were stratified according to their BMI (< or >25): the overweight (15 patients, BMI 27.4 ± 1.8) showed a lesser dialysis vintage compared to the normal weight group (21 patients, BMI 21.1 ± 2): 69.6 ± 49.9 months vs 128.9 ± 71.2; p = 0.045 and a higher score of histological severity (3.53 ± 1.0 vs 2.67 ± 1.1; p = 0.014). Pre-PTx serum P levels were significantly higher in overweight (7.17 ± 1.85 mg/dl vs 5.72 ± 1.08; p = 0.01). The overweight group kept significantly higher P levels at all time points (p = 0.01); the

overweight had the nadir P level at the fifth day post-PTx (4.6 mg/dl), the normal weight at the second day (3.4 mg/dl).
In conclusion, a higher BMI is associated with a more precocious and a more aggressive histological pattern of sHPH: this may be due to a different multicompartmental kinetics of P, possibly due to a larger P body burden.

W-PO40098

MANAGEMENT OF RENAL BONE DISEASE IN ACCORDANCE TO K/DOQI GUIDELINE-A SINGLE CENTER EXPERIENCE

CHEW THIAN FOOK[1], DR WONG HIN SENG[1], DR ZAKI MORAD[1]
Department of Nephrology, Hospital Kuala Lumpur, Malaysia[1]

Standard of care for renal bone disease in Department of Nephrology, Hospital Kuala Lumpur (HKL) is similar to other government heamodialysis centers (unpublished data). However, there is lack of such information in Ministry of Health heamodialyisis unit with respect to K/DOQI guideline.
Objectives: To study standard of care of renal bone disease with respect to K/DOQI guideline regarding corrected serum calcium (Ca^{2+}), serum phosphate (PO_4^{3-}), intact plasma parathyroid hormone (iPTH) and elementary calcium supplement from phosphate binders per day in year 2003 & 2004.
Design & Methods: 1. A cross sectional study was performed and involving patients receiving haemodialysis at HKL for more than 12 months duration in December 2003 & 2004 respectively. Demographic data, latest Ca^{2+}/PO_4^{3-}/iPTH level and calcium carbonate dosage per day were collected from clinical records. 2. Patients who had parathyroidectomy were excluded.
Results: A cohort of 105 patients was identified for the study in December 2003 & 2004. Majority is male gender, 66.7%. The duration of dialysis prior to study period was 104 ± 73 (28–303) months in December 2004. The iPTH/Ca/PO4/Elementary Ca results are summarized as below:

	2003 (%)	2004 (%)
iPTH 150–300 pg/dl	15.2	17.2
>300 pg/dl	54.3	55.2
<150 pg/dl	30.5	27.6
Ca 2.10–2.37 mmol/l	32.4	30.5
2.38–2.53 mmol/l	23.8	35.2
>2.54 mmol/l	34.3	27.6
<2.10 mmol/l	9.5	6.7
PO4 1.13–1.78 mmol/l	45.7	52.4
>1.78 mmol/l	43.8	36.2
<1.13 mmol/l	10.5	11.4
Elementary Ca <1.5 g/day	67.6	80
>1.5 g/day	32.4	20

Conclusions: Management of renal bone disease in HKL is still suboptimal and is similar to as reported in certain Europe center. Hyperphosphataemia and hypercalcaemia remain an issue in management of chronic dialysis population. However, it has improved since the introduction of K/DOQI guideline. Currently with the introduction of noncalcium phosphate binder and calcimimetic agents, there will be expected less incidence of hypercalcaemia and hyperphophataemia with better control of renal bone disease in future.

W-PO40099

FORECASTING TARGET RANGE OF INTACT PARATHYROID HORMONE IN HEMODIALYSIS PATIENTS: COMPARISON BETWEEN ARTIFICIAL NEURAL NETWORK AND NAIVE BAYES CLASSIFIER

JAINN-SHIUN CHIU[1], YUH-FENG WANG[1], YU-CHUAN LI[2]
Renal Informatics Lab, Department of Nuclear Medicine, Buddhist Dalin Tzu Chi General Hospital, Taiwan[1]*, Graduate Institute of Medical Informatics, Taipei Medical University, Taiwan*[2]

Objective: Measuring plasma intact parathyroid hormone (iPTH) concentration is crucial for managing renal osteodystrophy in hemodialysis (HD) patients. Although frequent measurements of plasma iPTH concentration are necessary to avoid inadequate prescription of vitamin D analogues, it is not cost-effective in most of the hospitals. For this purpose, we constructed and compared the performance of an artificial neural network (ANN) and a naive Bayes classifier (NBC) in forecasting target range of plasma iPTH concentration for HD patients.
Methods: The study population consisted of 130 HD patients (62 male and 68 female, age 59.72 ± 14.04 years). The predictors included demographic characteristics (gender, age), associated diseases (diabetes, hypertension), blood biochemistries (hemoglobin, protein, albumin, calcium, inorganic phosphorus, alkaline phosphatase, and ferritin), calcium-phosphorus product, and transferrin saturation. The outcome variable was dichotomous, either target group (150 pg/ml ≦ iPTH ≦ 300 pg/ml) or non-target group (iPTH < 150 pg/ml and iPTH > 300 pg/ml) based on K/DOQI guideline. A leave-one-out technique was adopted in cross-validation process. The ability of ANN and NBC to discriminate outcome was assessed using receiver-operating characteristics (ROC) curve analysis with the discriminating power determined by the area under the ROC curve (AUC).
Results: The final best ANN model used in this study was a feed-forward, multilayer perceptron network architecture using back-propagation algorithm. Pairwise comparison of each ROC curve showed that the ANN significantly outperformed the NBC (AUC = 0.899 ± 0.058 vs. 0.623 ± 0.076, $p = 0.003$).
Conclusion: ANN can accurately forecast target range of plasma iPTH concentration in HD patients and might be a useful tool to stratify the patients into target group.

W-PO40100

PTH-FRAGMENTS – CAP AND CIP AND BONE FORMATION MARKERS IN CHILDREN WITH RENAL FAILURE.

HELENA ZIOLKOWSKA[1], MARIA ROSZKOWSKA-BLAIM[1]
Department of Pediatrics and Nephrology Medical University of Warsaw[1]

Commercial intact PTH assays detect two immunoreactive fragments – whole PTH molecule (1–84 aminoacids PTH) and 7–84 PTH, which are biologically different. 1–84 PTH (cyclase activating PTH, CAP) increases, 7–84 PTH (cyclase inactive PTH, CIP) decreases bone turnover. The aim of the study was to estimate the effect of PTH and its fragments on bone formation markers in children with chronic renal failure (CRF). We examined 75 children with CRF: 41, aged 13.4 ± 4.3 on conservative treatment and 34 with end-stage renal disease. In all children serum level of calcium (sCa), phosphorus (sP), osteocalcin (OC), alkaline phosphatase (AP) were evaluated; intact PTH (PTHint) and CAP value were estimated with new Scantibodies laboratory immunoradiometric assay. The value of CIP, CAP/CIP ratio and percentage of CIP and CAP (%CIP and %CAP) were calculated. Correlations between examined parameters were calculated by the Spearman's correlation coefficients.
Significant correlations were found between OC and AP ($R = 0.5$, $p < 0.001$), PTHint ($R = 0.6$, $p < 0.001$), CAP ($R = 0.51$, $p < 0.005$), CIP ($R = 0.6$, $p < 0.001$) and sP ($R = 0.46$, $p < 0.001$). Significant negative correlation was found between OC and CAP/CIP ($R = -0.33$, $p < 0.01$). Significant correlation of PTHint with %CIP ($R = 0.38$, $p < 0.001$) and negative correlation with %CAP ($R = -0.38$, $p < 0.001$) were found.
Conclusion: The bone turnover expressed by osteocalcin level rises together with PTHint increase, despite of the increase of CIP fragment in PTH molecule. Increase of CIP fragments doesn't have negative influence on bone turnover expressed by osteocalcin level. The evaluation of significance CIP and CAP for bone formation in children with chronic renal failure require further studies.

W-PO40101

OSTEOPROTEGERIN (OPG) IN CHILDREN WITH CHRONIC RENAL FAILURE

HELENA ZIOLKOWSKA[1], MARIA ROSZKOWSKA-BLAIM[1]
Department of Pediatrics and Nephrology Medical University of Warsaw[1]

Glicoprotein, osteoprotegerin (OPG), a member of the TNF receptor superfamily is a natural inhibitor of osteoclastogenesis. OPG inhibits osteoclast activation and differentiation. OPG acts as a decoy receptor by binding the receptor activator of nuclear factor kB ligand (RANKL). The aim of the study was to estimate a concentration of OPG in children with chronic renal failure. Thirty-nine children, aged 13.6+/–4.5 years with chronic renal failure, 23 on conservative treatment (CRF), aged 13+/–3.5 years with creatinine clearance 47.6+/–20 ml/min/1.73 m^2 and 16 with end-stage renal disease (ESRD), aged 14.4+/–5.6 years were examined. In all children serum concentration of OPG, parathormone (PTHint), calcium (sCa), phosphorus (sP) and alkaline phosphatase (AP) were measured. OPG was determined by ELISA method (Biomedica), PTHint by RIA method, AP by kinetic method, sCa and sP were determined by standard laboratory biochemical assays. Correlations were calculated by the Spearman's correlation coefficients. As a normal concentration of OPG in children 4.05+/–1.63 pmol/l was accept (Buzi F et al. Clin Endocrinol 2004, 60:87–91).

Results:

	sCa (mmol/l)	sP (mmol/l)	AP (U/l)	OPG (pmol/l)	PTH (pg/ml)
CRF	2.45 ± 0.2	1.6 ± 0.3	190 ± 100	2.5 ± 1.8	69.5 ± 60
ESRD	2.4 ± 0.2	2.1 ± 0.5*	241 ± 180	2.9 ± 1.6	207 ± 170*

*p < 0.005 (Mann-Whitney test)

Significant correlations were found between concentrations of PTHint and OPG (R = 0.53, p = 0.003). No correaltion was found between OPG and sCa, sP and AP serum levels.
Conclusion: The higher levels of OPG in children with chronic renal failure and secondary hiperparathyroidism may play the protective role for bone. Further studies for evaluation of OPG function in bone metabolism in patients with chronic renal failure are needed.

W-PO400102

INTRAOPERATIVE IPTH ASSAY DURING TOTAL PARATHYROIDECTOMY WITH AUTOTRANSPLANTATION IN PATIENTS ON DIALYSIS

YOUNG-JOO KWON[1], JI EUN LEE[2], JAE BOK LEE[3], HEUI JUNG PYO[2]
Department of Nephrology, Korea University, Guro Hospita[1], Korea Univ. Guro Hospital[2], Dept. of Surgery, Korea Univ. Guro Hospital[3]

Background: Parathyroidectomy is indicated in the uncontrolled secondary hyperparathyroidism in patients on dialysis in spite of the various medical treatments. Rapid intraoperative PTH monitoring (IOPTH) has been known as the guide for minimally invasive excisions in primary hyperparathyroidism and the complete excision of functioning parathyroid glands in secondary hyperparathyroidism.
Objective: We evaluated whether IOPTH predicted the short-term (<1 year) postoperative iPTH levels.
Methods: We did total parathyroidectomy with immediate autotransplantation with IOPTH in nine patients on dialysis diagnosed as uncontrolled secondary hyperparathyroidism for sixteen months since November 2003. After removal of 4-parathyroid glands, we measured IOPTH at 10 and 20 minutes by biotinylated monoclonal Ab reacting with N-terminal fragment (1 ~ 37) and monoclonal Ab labeled with ruthenium complex reacting with C-terminal fragment (38 ~ 84) by Elecsys®2010. We measured the postoperative serum iPTH levels at 1-,3-,6- and 12-months.
Results: The mean preoperative serum iPTH level was 1223.2 + 374.5 pg/mL, level at 10 min and at 20 min decreased to 282.9 + 122.4 pg/mL (26.2 + 14.7% of preoperative level) and 200.3 + 123.7 pg/mL (18.2 + 13.8%) respectively. Postoperative iPTH level increased to preoperative levels in four patients with IOPTH level at 20 min over 15% of initial level.
Conclusion: We may recommend the decrement of IOPTH levels at 20 min less than 15% as possible in immediate autotransplantation. The accurate cut-off-level of IOPTH concerning the postoperative increment of iPTH levles remains to be proven in further studies.

W-PO40103

COMPARISON OF ULTRASOUND AND SCINTIGRAPHY FOR DETECTION OF PARATHYROID LESIONS

DWARAKANATHAN RANGANATHAN[1], ARAVIND RAVI KUMAR[2]
Renal Medicine, Royal Brisbane & Women's Hospital[1]; Nuclear Medicine, Royal Brisbane & women[2]

Aim: To evaluate scintigraphy and ultrasound for detecting parathyroid lesions.
Materials and Methods: A retrospective analysis of imaging of parathyroid glands in 20 consecutive patients who underwent both Ultrasound and Scintigraphy was performed. These patients were divided into two groups: Group I on dialysis suspected to have hyperplasia due to secondary hyperparathyroidism and Group II suspected to have adenoma with normal renal function. Scintigraphy was performed on modern dual head gamma cameras using the single tracer (Tc-99 m Sestamibi) dual phase method with SPECT. Ultrasound was performed using 7.5–10 MHz probes. Reports that were not clearly positive or negative were classified as indeterminate.

Results:

Calcium and PTH levels

	Group I		Group II	
	Mean	Median	Mean	Median
Calcium (2.15–2.60 mmol/l)	2.70	2.68	2.81	2.84
PTH (1.0–7.0 pmol/l)	147.10	181.50	26.33	12.00

Imaging

	Group I		Group II	
	MIBI Scan	US	MIBI Scan	US
Positive	5 * (50%)	2 (20%)	4 (40%)	2 (20%)
Negative	5 (50%)	3 (30%)	5 (50%)	4 (40%)
Indeterminate	0 (0%)	3 (30%)	1 (10%)	4 (40%)

*2 patients who had parathyroidectomy previously had ectopic mediastinal lesions detected by follow up scintigraphy only and in one patient implanted parathyroid tissue in forearm picked up by ultrasound only.

Nine of 10 patients in Group 2 had surgery. 8 Patients had adenoma histologically; 1 patient who had normal parathyroid tissue had negative scintigraphy and ultrasound.
Scintigraphy detected more parathyroid abnormalities and had less indeterminate results than ultrasound.
Conclusion: A prospective study involving larger number of patients is required to confirm our findings. The addition of thyroid subtraction scanning to Sestamibi scintigraphy and the routine use of Doppler ultrasound may lead to improved diagnosis.

Oxidative Stress / Inflammation

W-PO40104

INFLAMMATORY MARKERS AND HEPATOCYTE GROWTH FACTOR

CHIEN-TE LEE M.D.[1], CHING-YI YU[1], KING-KWAN LAM[1], JIN-BOR CHEN[1]
Division of Nephrology, Department of Medicine, Chang-Gung Memorial Hospital, Kaohsiung, Taiwan[1]

Dialysis hypotension is one of the important complications of hemodialysis. Of note, it is usually associated with increased morbidity and mortality. Multiple mechanisms are involved in the pathogenesis of dialysis hypotension. The role of chronic inflammation in dialysis-associated hypotension has not been investigated yet. Aims of our study are to study if patients with dialysis hypotension are more inflamed and the role of hepatocyte growth factor. A total of 40 patients with chronic hypotension were identified. Pre-dialysis serum inflammatory makers: high sensitivity C reactive protein (hs-CRP), interleukin-6 (IL-6), albumin, hepatocyte growth factor (HGF) as well as leptin, adiponectin levels were measured and compared with another 95 hemodialysis patients. Demographic data including age, gender, body mass index (BMI), dialysis duration were recorded. Our results found that there was no difference in inflammatory markers between non-hypotension and hypotension hemodialysis patients (hs-CRP: 4.9 ± 5.4 mg/L v.s. 5.8 ± 7.1 mg/L, IL-6: 4.4 ± 4.5 pg/mL v.s. 5.1 ± 6.3 pg/mL, both p > 0.05). The serum albumin, leptin and adiponectin levels were similar between two groups. Levels of serum HGF in patients with hypotension were not higher than those in non-hypotension group. The serum albumin levels were inversely correlated with hs-CRP (r = –0.392, p < 0.001) and IL-6 (r = –0.463, p < 0.001). Adiponectin was negatively correlated with hs-CRP (r = –0.225, p < 0.01). HGF also showed a positive relation with hs-CRP (r = 0.378, p < 0.001). No association was found between adiponectin and HGF. We conclude that chronic inflammation is prevalent in dialysis population. Serum HGF level is associated with inflammation but not with dialysis hypotension. Chronic inflammation appears to be irrelevant to hemodialysis-associated hypotension.

W-PO40105

SERUM FREE IRON AND OXIDATIVE STRESS IN HEMODIALYSIS PATIENTS NOT RECIEVING INTRAVENOUS IRON

RAVINDRA PRABHU A[1], MUNGLI PRAKASH[1], SHARMILA UPADHYA[1]
Kasturba Medical College, Manipal, India[1]

Background: Serum free iron or Non transferrin bound iron is increased in chronic renal failure patients receiving Hemodialysis and intravenous iron therapy. Whether this causes oxidative stress by generating hydroxyl radicals and lipid peroxidation is controversial.
We measured serum free iron in chronic renal failure patients not on intravenous iron and correlated it with serum ferritin and oxidative stress markers.
Materials and Methods: The study was conducted on three groups. Group A – healthy controls (n = 20). Group B – Predialysis chronic renal failure patients (n = 24). Group C – Chronic renal failure patients on maintainence hemodialysis not receiving IV iron or Erythropoetin (n = 22).
All subjects had Serum free iron both ferric and ferrous forms estimated by bathophenthroline disulphonate assay, oxidative markers i.e. protein thiols by spectrophotometry using dithiobisnitrobenzoicacid, lipid hydroperoxides by Fox II assay and serum ferritin by electrochemiluminiscence assay.
Mean values in the three groups were compared on SPSS using one way analysis of variance followed by multiple comparison post hoc test with application of Pearson correlation.
Results: Serum free Iron was significantly increased in Group C (Fe^{++} p = 0.008, Fe^{+++} p = 0.0001) as compared to Group A and B. There was no significant difference in levels of free iron between Group A and B.
Serum ferritin was significantly increased in Group C (p = 0.0001) as compared to Group A and B.
Lipid hydroperoxides were increased (p = 0.025, 0.001) and protein thiols reduced (p = 0.0001, p = 0.0001) significantly in both Group B and C as compared to Group A with no difference between Group B and C.
There was no correlation between free Iron and either protein thiols, lipid hydroperoxides or serum ferritin between the three groups.
Conclusion: Serum free Iron is increased in patients of chronic renal failure on hemodialysis not on intravenous iron and is catalytically inactive.

W-PO40106

MALNUTRITION AND INFLAMMATION IN HEMODIALYSIS

HELMUT M. WALTER[1], KIRSTEN S. BEIERLING-ROHRBACH[1]
University Erlangen-Nuernberg[1]

Malnutrition and chronic inflammation are predictors of vascular events and mortality in end-stage renal disease (ESRD). To determine the prevalence and interaction of these factors we studied prealbumin, serum albumin (SA), transferrin, cholesterol, nPCR, BMI, bioelectric impedance analysis, C-reactive protein (CRP) and other traditional vascular risk factors in 261 patients (152 men, 109 women) (38% diabetics) with different duration of hemodialysis (HD) treatment. 64% showed hints of protein-energy malnutrition (PEM) according to DOQI-guidelines. PEM was pronounced at the start of HD (especially in patients with nephrotic syndrome) and after long term (>5 y) HD treatment (independent of underlying disease). Diabetic patients seemed to be prone to PEM (72%). The degree of PEM did not correlate to the level of CRP (r = –0.21).
To test the hypothesis, that correction of malnutrition may modify inflammation, 30 patients with ESRD were followed for 5 months after starting HD treatment, randomised to either standard care or a dietary intervention (nutrition counselling up to nutrition supplements). There was a continuous fall of SA after start of HD in spite of normal levels of prealbumin. This decrease of SA could be prevented in the intervention group but there was no influence on CRP.
We conclude according to DOQI-guidelines PEM is frequently found in patients at start of (depending on nature of renal disease) and after long term HD therapy (>5 y). Due to frequent statine therapy hypocholesterinemia is not a good marker for malnutrition as well as transferrin due to the different levels of anemia. The short term parameter prealbumin seems not to reflect PEM sufficiently. Correction of PEM does not influence CRP over 5 month period. Therefore, SA seems to be the best marker for the definition of PEM. Dietary intervention is most important at the start and after long term (>5 y) HD therapy.

W-PO400107

UP-REGULATION OF DIETARY LONG-CHAIN FATTY ACIDS FOR LIPID METABOLISM IN HEMODIALYSIS

YUKIHIRO NISHIMOTO[1], AKEYO HORIE[1], JUNKO NISHIMOTO[2], YUMIKO NISHIMOTO[1]
Dept. of Nephrology and Dialysis Chukyo-Kosei Clinic Nagoya Japan[1], Div. of Internal Medicine Institute of Geriatric Healthcare Mantenboshi Nagoya Japan[2]

It has been reported that n-3 polyunsaturated fatty acids (PUFAs) are linked to reduction of the elongation of linoleic acid, suppression of n-6 PUFAs derived eicosanoids and carcinogenesis. The aim of this clinical trial was to investigate whether kinetics of n-3 and n-6 PUFAs metabolism were influenced by oral docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) in hemodialysis (HD) patients.
Plasma linoleic acid (18:2n-6), gamma-linolenic acid (18:3n-6), eicosadienoic acid (20:2n-6), dihomo-gamma-linoleic acid (20:3n-6), arachidonic acid (AA, 20:4n-6), docosatetraenoic acid (22:4n-6), linolenic acid (18:3n-3), EPA, docosapentaenoic acid (22:5n-3) and DHA were measured by gas chromatography after extraction and fractionation. The treatment group (n = 10) received 1 tablet containing 70 mg DHA, 24 mg EPA, 1.5 micro-gram vitamin C and 0.4 mg vitamin E daily. Samples were obtained before and after 8 weeks.
EPA levels (67.4+/–18.6 vs.103.7+/–31.4 micro-gram/ml, p = 0.028), EPA : AA ratio (0.56+/–0.15 vs. 0.82+/–0.23, p = 0.038) increased significantly. DHA levels were higher; 18:2n-6, 18:3n-6 and 20:3n-6 levels were lower after intervention. However, these showed no significant differences. The other levels were unchanged. n-1,5,7,9 PUFAs are under analysis.
DHA increase without significance suggests that DHA might be accelerated reversible to EPA and its oxidative modification to downstream. This requires further investigation, as the metabolic alteration of various tissues and organs in HD patients is unknown.

W-PO40108

OXIDATIVE STRESS IN PATIENTS UNDERGOING HAEMODIALYSIS

JUAN F MACÍAS NUÑEZ[1], MARTA PEREZ-PRIETO[1], JOSE M SANTIAGO[1], JAVIER ARRIETA[2], CARLOS FERNANDEZ-ANDRADE[3], CIPRIANO ESCAJA[4], GUILLERMINA BARRIL[5], JESUS BUSTAMANTE[6], ZUBIHAUR GHAIS[7], FELIPE RUBIO[1], ALFONSO OTERO[8], JOSE M LOPEZ-NOVOA[9]
Hospital Clinico Universitario de Salamanva[1], Hospital de Basurto, Bilbao[2], Hospital Universitario de Sevilla[3], FRIAT Bejar[4], Hospital de la Princesa, Madrid[5], Hospital Universitario de Valladolid[6], Hospital del Bierzo, Ponferrada[7], Hospital Cristal Piñor, Orense[8], Facultad de Medicina, Salamanca[9]

Oxidative stress and inflammation are major risk factors for cardiovascular morbid events and dead in patients in HD. HD with cuprophan increases oxidative stress (OS) whereas more biocompatible membranes (BM) do not modify OS. The purpose of this study has been to compare the effect of HD with PMMA-BKF with other HD membranes.
Methods: In 45 patients, (31 M and 14 F) with chronic renal failure undergoing HD for a mean of 16 months with their usual membranes (polysulfone, AN 69, or cellulose acetate), blood was withdrawn at the beginning (A) an at the end (B) of a HD session and immediately processed and stored for biochemistry. Then, all of them were shifted to PMMA-BKF for 6 months and new samples of blood were taken at the end of this period in the pre (C) and post dialysis (D) moments. The regime of HD, diet, time, body weight and pharmacological treatment remained unchanged for the 6 months of observation. OS results are shown in the Table.

	SOD (U/gHb)	GPx (UgHb)	GR (U/l)	TAS (mmol/l)	MDA (mmol/l)
A	606 ± 2	58.6 ± 1.2	62.1 ± 1.5	0.68 ± 0.02	1.78 ± 0.04
B	478 ± 14*	42.5 ± 1.9*	60.9 ± 2.2	0.52 ± 0.02*	2.04 ± 0.04*
C	857 ± 43* $	68.5 ± 1.7* $	62.2 ± 1.4	0.98 ± 0.04* $	1.63 ± 0.04* $
D	813 ± 58*$#	59.2 ± 2.3 $#	61.5 ± 1.9	0.78 ± 0.04*$#	1.83 ± 0.05 $#

*P > 0.05 vs A. $P > 0.05: vs B. #P > 0.05: vs C.

Additionally the anti-inflammatory IL 10 increased between A and C periods (from 12.77 2.43 to 16.58 4.56 pg/mL) whereas IL-6 decreased from 6.94 to 9.89 (P < 0.05). TNF-a and IL-1a did not change significantly. We conclude that chronic HD with PMMA-BK, compared with classical HD membranes, clearly improve OS after 6 months of HD. This may represent an advantage for the prevention and/or slow down of the development of arterioscleroses and dead induced by cardiovascular events.

W-PO40109

THE INFLUENCE OF POLYMORPHIC IL-6-174 AND IL-10-1082 GENES IN MALNUTRITION-INFLAMMATION SYNDROME ON HEMODIALYSIS PATIENTS

A SUHARDJONO[1], ENDANG SUSALIT[1]
Department of Internal Medicine, University of Indonesia, Jakarta, Indonesia[1]

Many studies on HD patients in developed countries have conferred strong evidence of closed correlation between inflammation, cardiovascular complication and high mortality rates. Adapting to high cost, HD in Indonesia is conducted in a little different ways. Patients are dialysed twice a week, 5 hours each, using reprocessed cellulose dialyzer, and without ultrapure water. All of these contribute to a high risk of inflammation, but in fact the prevalence of inflammation in Indonesia is relatively low. The purpose of this study was to obtain the frequency of polymorphic gene IL-6-174 and IL-10-1082 to find out the prominent factors in malnutrition inflammation syndrome.
We studied 81 HD patients, who had no infection, in stable condition and all of them used reprocessed diacetate cellulose dialyzers. It was obtained that CRP (6.23 ± 5.57 mg/L), malnutrition prevalence (SGA B, C 24.7%), and malnutrition inflammation score (6.7) were lower compared with the data from Europe and the United States. Out of 64 patients examined, IL-6-174GG was obtained 95.31%, GC 1.56% and CC 3.13%, IL-1082AA 89.06%, GA 10.94%, but absence of GG genotype. The proportion of these alleles was almost similar to that obtained in Korea, Japan and China, but it was different from that obtained in the US for the Caucasian race, African Americans, Hispanic people, and the Caucasian people in Europe. The low prevalence of inflammation and lower level of CRP may be related to very low IL-6-174C allele. A very resolute impression was obtained in our HD patients that IL-6-174GG gene was protective in nature whereas IL-10-1082 AA gene had a less considerable role.

W-PO40110

THE EFFECTS OF ATORVASTATIN ON OXIDATIVE STRESS IN HAEMODIALYSIS PATIENTS

IOANNIS CHOULIARAS[1], GEORGE D DIMITRIADIS[1], THEODORE G THEODORIDIS[2], VASSILIKI TH GALEA[2], GEORGE L CHOULIARAS[1], CHRYSTALLENI T CHRISTODOULIDOU[1], NIKOLETTA N NIKOLOPOULOU[2], VASSILIKI D COKKINOU[1], VALSAMAKIS E HADJICONSTANTINOU[3]
Nephrology, Evangelismos General Hospital, Athens, Greece[1]*, Hematology, Evangelismos General Hospital, Athens, Greece*[2]*, Evangelismos General Hospital, Athens, Greece*[3]

ESRD patients on haemodialysis (HD) experience increased morbidity and mortality mainly due to cardiovascular disease (CVD). Among numerous CVD risk factors, oxidative stress seems to hold a key role in large scale prospective studies concerning general population and HD patients. Statins [3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors] have been shown to exert beneficial effects in preventing CVD, beyond their well established lipid-lowering action, by means of their so called pleiotropic effects. Among the latter, prevention of oxidative injury is currently being given emphasis as an emerging, non traditional CVD risk factor.
We examined the influence of atorvastatin on redox status in 14 HD patients, by means of serum total antioxidant capacity (TAC) determination. Their age had a median value of 60.5 years and they were under HD for more than two years.
TAC was determined by the colorimetric test system Imanox (Immundiagnostik, Germany) before and 12 months following 10 mg daily atorvastatin treatment. TAC levels (presented as mean ± SE) were significantly increased after the 12 month period (321.5 ± 12 μmol/L at 12 months vs 281.5 ± 7.2 μmol/ L at baseline, $p = 0.0036$ by paired t-test and $p = 0.007$ by Wilcoxon matched pairs test).
In our study atorvastatin use was associated with significant elevation of serum TAC levels, probably attritutable to the emerging pleiotropic effects of statins, namely their antioxidative and anti-inflammatory properties. These preliminary data support the use of atorvastatin in HD patients beyond the classic lipid lowering action, by means of an inhibitory effect on oxidative stress.

W-PO40111

IMPACT OF NUTRITIONAL STATUS ON OXIDATIVE STRESS IN HEMODIALYSIS PATIENTS WITH/WITHOUT INFLAMMATORY STATE

SIREN SEZER[1], EMRE TUTAL[1], AYSE BILGIC[1], DERYA ALDEMIR[2], SUNA TURKOGLU[2], ZUBEYDE ARAT[1], FATMA NURHAN OZDEMIR[1], MEHMET HABERAL[3]
Baskent University Faculty of Medicine, Department of Nephrology[1]*, Baskent University Faculty of Medicine, Department of Biochemistry*[2]*, Baskent University Faculty of Medicine, Department of General Surgery*[3]

Hemodialysis (HD) patients are under risk of increased oxidative stress, a factor that leads to higher rates of complications. We aimed to evaluate the interactions between oxidative stress and nutritional parameters of HD patients from the perspective of chronic inflammation presence.
98 patients (35F/63M, aged; 51.3 ± 13.9 years, HD duration; 78.5 ± 52.1 months) were included. Indicators for the oxidative status of plasma were measured [superoxide dismutase (SOD), glutathione peroxidase (GPX) and malonyldialdeheid (MDA)]. Last one year's monthly determined laboratory (CRP, albumin, calcium, phosphorus, iPTH, lipid parameters, homocysteine, Lp(a), predialysis BUN, creatinine, nPCR] and clinical findings (Pre/post dialysis blood pressures, cumulative iron therapy doses) were recorded and analyzed retrospectively. Patients were classified as; with (Group I, $n = 30$) or without (Group II, $n = 68$) a chronic inflammation according to mean CRP levels. Mean plasma levels for SOD, GPX and MDA were 1009.0 ± 493.5 U/gHb, 44.3 ± 15.2 U/gHb, 10.5 ± 2.1 nmol/mL respectively. In group I, increased oxidative stress (increased MDA levels) was negatively correlated with dialysis duration ($p < 0.02$) and positively correlated with age ($p < 0.01$) while decreased oxidative stress (increased SOD/GPX levels) was positively correlated with pre-dialysis BUN ($p < 0.02$), nPCR ($p < 0.02$), homocysteine ($p < 0.01$), total cholesterol ($p < 0.001$), LDL-cholesterol ($p < 0.01$), HDL-cholesterol ($p < 0.05$), triglyceride levels ($p < 0.001$) and negatively correlated with pre-/post-dialysis systolic blood pressures ($p < 0.01$). In group II, increased oxidative stress was positively correlated with total cholesterol ($p < 0.01$) and pre- and post-dialysis systolic blood pressures ($p < 0.001$) while increased antioxidative capacity was negatively correlated with predialysis BUN ($p < 0.01$), ferritin levels ($p < 0.01$), cumulative iron therapy doses ($p < 0.01$) and positively correlated with Kt/V ($p < 0.01$).
As a conclusion, in patients with chronic inflammation, nutritional status interferes with oxidative stress. In this case, good nutritional status turns to an advantage of increased antioxidative capacity and therefore a favorable cardiovascular outcome. In patients without chronic inflammation higher dialysis adequacy seems to be the major determinant of antioxidative capacity.

W-PO40112

OXIDATIVE MODIFICATIONS OF BIOLOGICAL PROPERTIES OF SERUM ALBUMIN IN HEMODIALYSIS PATIENTS

PAIK SEONG LIM[1]
Tung Taichung Metroharbour Hospital[1]

Epidemiological data consistently suggested that cardiovascular disease is the major cause of mortality in end-stage renal disease (ESRD) population. Several lines of evidence have suggested that ESRD appears to be in a state of enhanced oxidative stress. Human serum albumin (HSA), the major transport protein in blood plasma, has the greatest potential as an extracellular antioxidant. The present work aimed to investigate the biological alterations uremic HSA. Our data showed that uremic-related HSA was characterized by changes in absorption and fluorescence spectra as well as exhibited increased levels of dityrosine, carbonyl groups, and decreased thiols. We have also observed characteristic change in electrophorectic mobility of the HSA that may affect its free radical scavenging capability. Using the copper-mediated oxidation of human LDL and free radical-mediated hemolysis test, we found that uremic HSA has lost some of its protective antioxidant properties. The present study further supports the notion that, in addition to its serum concentration, the quality and integrity of HSA molecule may be a crucial factor affecting its protective effects. Since increased oxidative stress in ESRD population may contribute to its cardiovascular complications, it seems likely that oxidative alteration of HSA might be an important risk factor.

W-PO40113

RELATIONSHIP BETWEEN SERUM PROHEPCIDIN AND FERRITIN CONCENTRATIONS IN HEMODIALYSIS PATIENTS WITH CHRONIC KIDNEY DISEASE (CKD)

ANDRZEJ WIECEK[1], ANTONI WYSTRYCHOWSKI[1], WOJCIECH MARCINKOWSKI[1]

Dept. of Nephrology, Endocrinology and Metabolic Diseases, Medical University of Silesia, Katowice, Poland[1]

Background: Hepcidin (H), 25-aminoacid peptide secreted by the liver in response to infection and inflammation, inhibits iron absorption from the gastrointestinal tract and iron release from reticuloendothelial system. Uremia is associated with increased oxidative stress, aggravated by hemodialysis procedures, infections and intravenous iron infusions. The aim of this study was to assess serum level of prohepcidin – hepcidin prohormone in hemodialysis patients with CKD. Additionally, we evaluate relationships between serum prohepcidin concentratiion and degree of anemia, as well as iron metabolism parameters in these patients.

Material and methods: With U-Mann-Whitney test we compared serum prohepcidin concentration (ELISA kit) in 83 hemodialysis patients and in 20 healthy volunteers. Spearman rank correlation analysis was used to assess whether serum prohepcidin level in hemodialysed patients is related to hematocrit (Ht) or iron metabolism parameters: serum iron (Fe) and ferritin concentrations, total iron binding capacity (TIBC) and transferrin saturation (TSAT).

Results: Serum prohepcidin concentration was significantly higher in hemodialysis patients than in healthy subjects (230 ± 60 vs. 171 ± 35 ng/ml; $p < 0.0001$). As shown in table, there was significant positive correlation between serum levels of prohepcidin and ferritin.

	Number of patients	Spearman's coefficient	*p* value
Ht	82	0.09	0.4
Fe	80	0.20	0.08
TIBC	82	–0.17	0.13
TSAT	78	0.22	0.05
ferritin	78	0.54	<0.0001

Conclusion: Serum prohepcidin level is significantly elevated in CKD patients on hemodialysis treatment and is related to serum ferritin concentration. This relationship seems to characterize patients with inflammation/infection.

W-PO40114

PLASMA SULFHYDRYLS AS A BIOMARKER OF OXIDATIVE STRESS IN CKD PATIENTS

BODIL A.K. SJÖBERG[1], MOHAMED SULIMAN[1], BJORN ANDERSTAM[1], ANDERS ALVESTRAND[1]

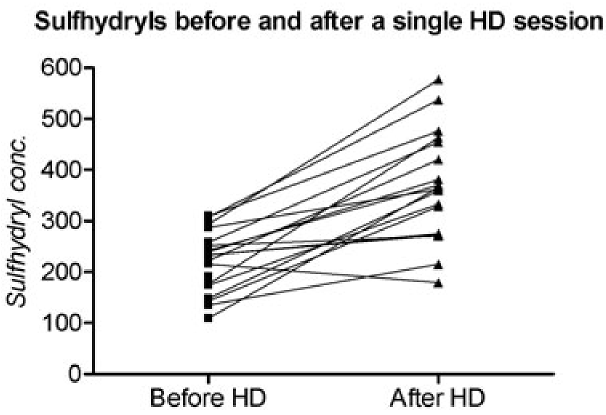
Dept. of Clinical Science, Div. of Renal Medicine, Karolinska Institutet, Stockholm, Sweden[1]

Introduction: Patients with chronic kidney disease (CKD) have a high incidence of cardiovascular disease. Oxidative stress is implicated in the development of atherogenesis. The concentration of free sulfhydryl groups is an established marker for oxidative stress. The aim of this study was to determine the plasma concentration of sulfhydryls in CKD patients and its association with renal function and HD treatment.

Methods: The characteristics of patients and controls are given in table below. All subjects were studied after an overnight fast; HD-pts before and after a single 4 h HD session using polyamine membranes (Polyflux 17L, Gambro). Plasma free sulfhydryl-group concentration was assayed spectrophotometrically according to the method by Ellman.

Group	HD	PD	CKD	Control
Number	19	12	47	15
Age (yrs)	66 ± 10	58 ± 16	57 ± 17	62 ± 16
GFR (ml/min)			23 ± 12	85 ± 18
Sulfhydryl (µmol/l)	219 ± 60	256 ± 89	254 ± 97	341 ± 61

Results: The mean sulfhydryl concentrations were significantly decreased in HD, PD and nondialyzed CKD patients compared with controls. A single HD treatment normalized sulfhydryl conc. (before HD 219 ± 60, after HD 368 ± 106 µmol/l; $p < 0.001$). Sulfhydryl conc. correlated positively with p-albumin ($\rho = 0.55$; $p < 0.0001$) and Hb ($\rho = 0.36$; $p < 0.001$) and negatively with p-urea ($\rho = -0.38$; $p < 0.0001$) and s-creatinine ($\rho = -0.28$; $p < 0.01$). In nondialyzed CKD patients and controls, sulfhydryl concentrations correlated with GFR ($\rho = 0.32$; $p < 0.01$). After correction of sulfhydryls for p-albumin, stepwise multiple regression showed highly significant correlations between sulfhydryls and p-urea for all patients ($p < 0.001$) and the nondialyed CKD patients ($p < 0.002$).



Conclusions: In CKD patients, sulfhydryl conc. are decreased, indicating increased oxidative stress, and correlate with p-urea conc. A single HD can normalise the sulfhydryl concentration and improve the oxidative status, which, at least partly, depends on the urea concentration.

W-PO40115

RELATIONSHIP BETWEEN SERUM PARAOXONASE ACTIVITY, CYSTATIN C AND HOMOCYSTEIN LEVELS IN HEMODIALYZED AND TRANSPLANTED PATIENTS

ILDIKO SERES[1], L.LOCSEY[2], A.DAN[3], B. BORBAS[2], L.ASZTALOS[4], GY. PARAGH[1],

First Department of Medicine, Medical and Health Science Center, University of Debrecen, Hungary[1]*, EuroCare 10 AKD*[2]*, Central Laboratory, Kenezy Hospital, Debrecen*[3]*, Department of Surgery, Medical and Health Science Center, University of Debrecen, Hungary*[4]

Introduction: Kidney transplanted and uraemic patients have an increased risk of atherosclerosis, increased lipoprotein oxidation. The serum paraoxonase (PON) is a HDL-associated hydrolase, which inhibits LDL oxidation. In uremic, dialyzed patients have hyperhomocysteinemia, which independent risk factor for accelerating atherosclerosis and cardiovascular complications. The aim of our study is to determine the serum paraoxonase activity, phenotype distribution and dyslipidemia in relationship with cystatin C, homocystein levels in renal transplanted, dialyzed patients compared to the values of healthy controls.

Patients and Methods: 110 healthy controls, 115 renal transplanted (43.5 ± 6.9 ys) and 112 hemodialyzed patients (61.3 ± 8.1 ys) were enrolled in the study. The PON activity was measured spectrophotometrically, PON phenotype was determined with dual substrate method. The total homocystein (FPIA), cystatin C concentration were determined with PETIA method.

Results: In kidney transplanted patients the PON activity was higher than dialyzed patients (121.1 ± 78.76 vs. 87.35 ± 54.72 U/L, $p < 0.01$). The different immunosuppressive treatment did not influence PON activity. Transplanted patients have high total cholesterol, LDL-C and apoB levels. Dialyzed patients have hypertriglyceridemia associated with low HDL-C and apoA1 levels. The homocystein level was significantly higher in dialysed patients compared to transplanted (25.52 ± 6.71 vs.15.46 ± 5.02; $p < 0.01$). The cystatin C level was highest in the dialyzed patients. The serum homocystein and cystatin C levels increased with renal failure progression in chronic rejection. The PON activity showed negative correlation with homocystein and cystatin C levels in dialyzed and transplanted patients. The standardized PON activities (PON/HDL; PON/apoA1) were lowest in dialyzed patients.

Conclusion: The PON activity decreased in uremic, dialyzed patients and showed negative correlation with cystatin C and homocystein levels. After kidney transplantation there was a significant increase in PON activity and decrease in homocystein and cystatin C level. These results suggest that low PON activity may contribute the deterioration of kidney function and the progression of atherosclerosis.

W-PO40116

PROFOUND ACCUMULATION OF NOVEL ADVANCED GLYCATION FREE ADDUCTS IN URAEMIA AND THEIR CLEARANCE BY DIALYSIS

ANNE DAWNAY[1], STAMATINA AGALOU[2], NAILA AHMED[2], PAUL J THORNALLEY[2]
University College Hospital[1], University of Essex[2]

Advanced glycation end products (AGEs) accumulate in uraemia and diabetes and have been implicated in the pathogenesis of cardiovascular disease. Their free adducts are a class of uraemic toxin of low mass (<400 Da) and we have characterised these in non-diabetic patients on haemodialysis (HD, n = 8) and peritoneal dialysis (PD, n = 8) compared with 8 healthy controls. AGE free adducts N-carboxymethyl-lysine (CML), N-carboxyethyl-lysine (CEL), the hydroimidazolones derived from methylglyoxal (MG-H1), glyoxal (G-H1) and 3-deoxyglucosone (3DG-H), methylglyoxal lysine dimer (MOLD), argpyrimidine (AP) and pentosidine (P) were determined in plasma, urine and dialysate ultrafiltrates by LC-MS/MS. In HD (pre) & PD compared with control plasma, there were significant elevations of CML (221 ± 11 & 111 ± 18 vs 19 ± 3 nM, p < 0.001), CEL (740 ± 75 & 336 ± 64 vs 35 ± 6 nM, p < 0.001), G-H1 (256 ± 17 & 149 ± 23 vs 40 ± 7 nM, p < 0.001), MG-H1 (4824 ± 429 & 2236 ± 592 vs 122 ± 23 nM, p < 0.001), 3DG-H (1230 ± 234 & 965 ± 195 vs 122 ± 15 nM, p < 0.001) and P (4.2 ± 0.6 & 5.1 ± 0.4 vs 0.9 ± 0.5 nM, p < 0.001) but MOLD in HD only (9.5 ± 1.0 vs 3.0 ± 0.7 nM, p < 0.001) and not AP. Most adducts rapidly equilibrated into dialysate and HD for 4 h decreased all plasma adducts up to 6-fold but only normalised MOLD and P. Mean reduction ratios ranged from 52 to 84% compared with 69% for urea. In PD, combined renal and peritoneal clearance of most free adducts was significantly lower than control renal clearance whereas mean total 24 h excretion rates were elevated up to 9-fold (MG-H1), the majority via the peritoneum. D/P ratios at 4 h of the PET commonly exceeded 1. We conclude that uraemia causes profound abnormalities in a range of plasma AGE free adducts due to impaired clearance and increased synthesis from elevated precursors in plasma. Increased synthesis probably as a consequence of glucose degradation products in PD fluid likely contributes to the elevated daily excretion in PD patients.

W-PO40117

DEPRESSION AND INFLAMMATORY MARKERS: COMPARISON BETWEEN HAEMODIALYSIS AND PERITONEAL DIALYSIS

SANJA SIMIC-OGRIZOVIC[1], DIJANA JOVANOVIC[1], TAMARA CAVIC[2], MILAN RADOVIC[1], MILAN STOSOVIC[1], VIOLETA DOPSAJ[3], ZORICA SUMARAC[3], VIDOSAVA NESIC[1]
Institute of Urology and Nephrology, Clinic of Nephrology, CCS, Belgrade, Serbia and Montenegro[1], Clinic of Psychiatric[2], Institude of Biochemistry[3]

Recent studies have suggested a relationship between depression, the major psychological problem in end stage renal disease (ESRD) patients (pts), and inflammation. The aim of the study was to compare depression incidence rate (DIR) in hemodialysis (HD) and peritoneal dialysis (PD) pts and relationship between depression and markers of inflammation in two ESRD treatments. The study included 107 HD pts (aged 55.3 ± 13 years) and 84 PD pts (aged 57.2 ± 13 years), maintained by HD (97.2 ± 66.5 month) or PD (14.4 ± 15.6 month) at our Institute. They were screened for depression by using the self-administered Beck Depression Inventory (BDI) questionnaire (score > or = 11 indicated depression). Plasma interleukin-6 (IL-6) and IL-10 levels, measured by ELISA and C-reactive protein (CRP) by turbidimetric method, were used as inflammatory markers. HD pts compared to PD pts were significantly younger and longer on dialysis, had significantly lower DIR (45.8% vs. 57.1%) and non-significance lower BDI score (18.3 ± 5.9 vs. 21 ± 10.2). After adjusted all pts for age and dialysis duration, 78 HD and 51 PD pts were divided into 4 groups according to BDI score: 2 depressed (DHD and DPD) groups and 2 non-depressed (nDHD and nDPD) groups. IL-6 (6.6 ± 6.9 vs. 3.5 ± 2.34 pg/ml and 11.5 ± 15.7 vs. 4 ± 5.4 pg/ml) levels were significantly higher in both depressed groups as well as CRP (7.9 ± 9.7 vs. 5.9 ± 6.5 mg/L and 26 ± 10.8 vs. 4.5 ± 2.5 mg/L) compared to non-depressed groups. IL-6 and CRP levels were lower in DHD compared to DPD group but without significance. In multivariate analysis BDI score was associated with IL-6 (p = 0.021; p = 0.013) levels in HD and PD pts. In conclusion, the HD and PD pts are very depressed and in both ESRD treatment BDI score was strongly associated with chronic inflammation. Although, in HD pts the DIR is lower and HD pts had lower proinflammatory markers compared to PD pts.

W-PO40118

WHOLE BLOOD GLUTATHIONE, ERYTHROCYTE REDOX STATUS AND PLASMA SELENIUM IN CHRONIC RENAL PATIENTS AND PATIENTS ON CHRONIC HAEMODIALYSIS THERAPY: EFFECT OF TWO DIFFERENT MEMBRANES

MAMOUN ELAWAD[1], TREVOR THOMAS[1], ROBERT WILKINSON[1]
Renal Medicine, University of Newcastle Upon Tyne, UK[1]

Chronic renal failure (CRF) and haemodialysis (HD) patients manifest increased oxidative stress (OS), due to imbalance between formation of reactive oxygen species (ROS) and antioxidants. Erythrocyte redox status (E. GSSG/GSH) is one of the biomarkers of increased OS. Plasma selenium (PSe) is a major antioxidant. In this study whole blood (WBGX) and plasma total and oxidised glutathione (GSSG) were spectrophotometrically determined in 15 normal controls (NC), 9 CRF patients and 16 patients on bicarbonate haemodialysis using cuprophane membrane (membrane A). Erythrocyte reduced glutathione (GSH) and GSSG were calculated from the data because these cells are the major compartments of blood glutathione and their survival may be shortened by oxidant damage. PSe was measured using atomic absorption spectrometry equipped with a graphite furnace. These measurements were repeated in 9 of the HD patients after at least 4 months on a polysulfone membrane (membrane B) which is more biocompatible than membrane A. Results (mean ± SEM) are given in the table below.

	WBGX (µmol/l)	E. GSSG/GSH	PSe (µmol)
NC	981 ± 41	0.001	1 ± 0.03
CRF	456.7 ± 20**	0.0047**	0.48 ± 0.1*
Prehaemodialysis A	503 ± 31**	0.0025**	0.55 ± 0.08*
Posthaemodialysis A	599 ± 38**	0.0037*	0.75 ± 0.08*
Prehaemodialysis B	677 ± 44**	0.001	0.79 ± 0.05*
Posthaemodialysis B	859 ± 71	0.0024**	1 ± 0.05

*P < 0.05, **P < 0.001 (Compared to NC)

PSe was positively correlated to WBGX [r = 0.76, p = 0.00] and negatively correlated to E. GSSG/GSH [r = –0.66, p = 0.00].
Conclusion: The data showed that CRF and HD patients are under increased OS. Both types of membranes induced acute OS stress but less in the long term with polysulfone membrane, probably due to its biocompatibility. CRF and HD patients have significantly lower PSe. PSe was significantly associated with better antioxidant defences and reduced OS.

W-PO40119

ANTIBODIES AGAINST β2GPI AND A CD40 PEPTIDE IN DIALYSIS PATIENTS

ANTHI BALITSARI[1], MICHAEL SAMARKOS[2], JOHN BOLETIS[1], PANAYIOTIS VLACHOYIANNOPOULOS[3]
Department Of Nephrology Laikon Hospital Greece[1]; 5th Department Of Internal Medicine Evangelismos Hospital Greece[2]; Department Of Pathophysiology Medical School National University Of Athens Greece[3]

Background: The impaired vasculature of patients receiving chronic dialysis therapy crucially contributes to the poor outcome. Antiphospholipid antibodies are considered one of the factors contributing to endothelial dysfunction which is a common pathophysiologic pathway leading to cardiovascular disease. The aim of the study was to investigate a possible correlation of antibodies against β2GPI (anti-β2GPI) and antibodies against the 239–245 peptide of CD40 (anti-CD40pep) with hypertension, left ventricular hypertrophy (LVH) and coronary heart disease, in patients undergoing hemodialysis. The 239–245CD40 peptide was chosen because of its sequence homology with the 7–13 peptide of β2GPI. We also recorded the presence of diabetes mellitus, as a cardiovascular disease risk factor.
Methods: We studied 30 patients undergoing hemodialysis for at least one year. We measured anti-β2GPI and anti-CD40pep with ELISAs, while left ventricular hypertrophy was assessed by echocardiogram.
Results: 13/30 patients (43.3%) were positive for anti-β2GPI and 12/30 patients (40%) were positive for anti-CD40pep. 9/30 (30%) patients presented LVH, 14/30 (46.6%) had hypertension, 5/30 (16.6%) had coronary heart disease, while 3/30 (10%) had diabetes mellitus.Anti-CD40pep positivity was strongly associated with the presence of hypertension (Fisher s exact test, p = 0.002) and LVH (p 0.05). Similarly there was no correlation between titers of anti-CD40pep and for anti-β2GPI (Spearman rho, p > 0.05).

Conclusions: Anti-β2GPI and anti-CD40pep were detected in a significant number of patients undergoing dialysis. In these patients there was an association between positivity for anti-β2GPI or anti-CD40pep and the presence of hypertension and LVH. It is possible that these antibodies contribute to cardiovascular disease by activating the vascular endothelium.

Anemia Management

W-PO40120

INJECTION PAIN IS INCREASED WITH DARBEPOETIN-α COMPARED TO EPOETIN-β

CP SCHMITT[1], BRUMMER C[2], ROSENKRANZ J[2], SCHAEFER F[2]
University Children's Hospital[1], University Children[2]

Darbepoietin-alpha (Aranesp) is a modified erythropoietin (EPO) with longer half-life, applicable at longer intervals. Our early experience in children on PD suggested increased injection pain with Aranesp relative to Epoietin-β (NeoRecormon), possibly related to technical differences, patient anxiety or the injected fluids per se.

13 patients with ESRD and EPO-dependent anemia, aged 3 to 22 years, received 3 injections of Aranesp or NeoRecormon in randomized order at 4 week intervals. The injected doses were calculated to substitute the usual weekly (n = 6) or biweekly (n = 7) doses (200 IU NeoRecormon/week = 1 μg Aranesp/week). They were filled into neutral syringes, diluted if necessary with saline to the same volume (0.6 ml) and injected with a 27G needle. Patients, parents and the nurse performing the injections were blinded as to the nature of the compound injected. Pain perception was recorded immediately and 30 min after injection on a visual analogue scale (VAS, 0 = no pain, 10 = maximal possible pain; complemented by 5 'smilie' faces).

The patients perceived more intense immediate injection pain with Aranesp than with NeoRecormon (5.4 ± 1 vs. 2.3 ± 0.6, p = 0.02). This was confirmed by the impression of the parents (5.3 ± 1 vs. 2.0 ± 0.9, p = 0.03) and the nurses (4.4 ± 1 vs. 2.2 ± 0.6, p = 0.02). Injection pain was inversely related to patient age (r = –0.53, p = 0.006). Interestingly, the difference in injection pain was bimodally distributed, with no or mild differences in 6, but a marked difference perceived in 7 patients (³4 VAS points). After 30 min, the injection site was largely painless with both drugs. No significant local reactions occurred with either medication (0.3 ± 0.1 vs. 0.3 ± 0.1 on a 5 score scale).

Subcutaneous injections of Aranesp are more painful than those of NeoRecormon in the majority of pediatric patients. The difference in painfulness is not explained by differences in injected volume or needle properties, and may limit the subcutaneous applicability of Aranesp.

W-PO40121

OPTA – OPTIMAL TREATMENT OF RENAL ANAEMIA: IRON AS A MAJOR FACTOR INFLUENCING TREATMENT OUTCOME

ROLAND M. SCHAEFER[1], IAIN C MACDOUGALL[2], JEROME ROSSERT[3], WALTER H HOERL[4]
University of Muenster[1], Department of Renal Medicine, King[2], Department of Nephrology, Hopital Tenon, Paris, France[3], Department of Nephrology, Allgemeines Krankenhaus Wien, Vienna, Austria[4]

Iron supplementation is one of the major factors influencing the efficacy of renal anaemia therapy. Optimal iron substitution is necessary to reach the recommended target haemoglobin of 11 g/dl in all patients (EBPGs; K/DOQI) as well as to optimize Epoetin dosage and treatment costs.

Data from DOPPS and the current European Survey of Anaemia Management (ESAM 2003) have shown that 47% (DOPPS) and 33.9% (ESAM 2003) of dialysis patients had not reached the target haemoglobin of >11 g/dl (23% / 13.0% had a haemoglobin <10 g/dl). 26.6% of patients in ESAM 2003 were iron-deficient. 17.5% suffered from functional iron deficiency and 9.1% had signs of absolute iron deficiency.

The OPTA Working Group for Iron gives the following recommendations on iron supplementation in order to increase haemoglobin levels in hyporesponsive patients and to guarantee cost-effective usage of Epoetins.

Absolute iron deficiency: Serum ferritin <100 ng/ml, should be distinguished from **functional iron deficiency:** Ferritin >100 ng/ml, transferrin saturation <20%. Monitoring iron status and controlling iron supplementation should be done in stable patients by measuring ferritin and transferrin saturation at 3-monthly intervals. Ideally, hypochromic red cells should be determined whenever possible.

Target levels of iron parameters:	Optimal	Acceptable
Ferritin (ng/ml)	200–500	100–800
Transferrin saturation (%)	30–40	20–50
Hypochromic red cells (%)	<2.5	<10

Numerous publications have shown both an increase in haemoglobin and simultaneous reductions in Epoetin dosage (30–70%) when these targets were met (Schaefer, 1992, Hoerl, 1995, Macdougall, 1996).

To compensate for blood loss in dialysis patients and to ensure sufficient iron stores during correction phase of anaemia, intravenous administration of iron is mandatory.

In conclusion, optimal iron substitution is essential to reach recommended target haemoglobin levels in dialysis patients and to optimize treatment efficacy. The OPTA recommendations have been developed to improve the transfer of the European Best Practice Guidelines into daily practice and to reduce the high number of patients with iron deficiency.

W-PO40122

INTRAVENOUS IRON SUCROSE (VENOFER®) ALONE IS CAPABLE TO CORRECT ANAEMIA IN PATIENTS WITH CHRONIC KIDNEY DISEASE NOT ON RENAL REPLACEMENT THERAPY

ROLAND M. SCHAEFER[1], KHASABOV, N.N.[2], ERMOLENKO, V.M.[3], GUREVICH, K.Y.[4], SCHREIBER, R[5], SHILO, V.Y.[6], TOMILINA, N.A.[7]
University of Muenster[1], Pirogov Medical Center, Moscow[2], Botkin Clinical Hospital, Moscow[3], Clinic of Extracorporeal Hemocorrection, St. Petersburg[4], Dialysis Center, Coesfeld, Germany[5], City Hospital #20, Moscow[6], City Hospital #52, Moscow[7]

Objective: Evaluation of the efficacy and safety of iv iron sucrose (Venofer®) alone versus iv iron sucrose plus epoetin beta (Epoetin) in anaemic patients with chronic kidney disease (CDK) not on renal replacement therapy.

Study design: Open-label, randomized study in 50 anaemic CDK patients (Hb: 8–11.5 g/dl) requiring iron supplementation. Correction phase: Group A: 5 infusions of 200 mg iron sucrose weekly (day 1–29). Group B: In addition to iv iron 4000 IU of Epoetin beta sc weekly (day 1–36). Maintenance phase: Group A and B: 100 to 200 mg of Venofer® i.v. monthly (day 43–169) to maintain Hb at 11–12.5 g/dl, TSat at 30–50%, and ferritin between 300 and 500 ng/ml. Group B: Epoetin continued. Group A: Epoetin added if Hb < 9 g/dl. The primary endpoint was the change in Hb (baseline to day 43).

Results: These preliminary results show that there was clear-cut improvement in ferritin and TSat during the correction phase (day 1–43) in both groups. The 95% confidence interval of change in Hb from baseline was 0.2 to 1.1 g/dl in Group A and 1.2 to 2.1 g/dl in Group B, indicating significant correction of anaemia in both groups. Hb levels were constant during maintenance phase, Hb values in Group A remained above 11 g/dl. Ferritin values never rose above 800 ng/ml. So far no serious adverse events related to iv iron sucrose have occurred in this trial.

Conclusions: At the end of the correction phase iron indices and Hb had improved significantly in both groups, indicating that anaemic CDK patients not on renal replacement therapy benefit from iv iron sucrose. The improvement was maintained in both groups after the correction phase. Thus, in CDK patients iv iron sucrose alone is capable to correct renal anaemia and to maintain Hb levels above 11 g/dl. Given the high cost of Epoetins, this outcome may be of great economical significance.

W-PO40123

THE USE OF ANDROGENIC STEROID IN PD PATIENTS

KWOK HONG CHU[1], FUNG KA SHUN[1], TANG HON LOK[1], TONG KWOK LUNG[1], CHAN WAI HAN[1], TSANG WAI KAY[1]
Princess Margaret Hospital[1]

Objectives: Nutrition and anaemia are two prominent prognostic indicators in dialysis patients. Synthetic erythropoietin therapy is effective but expensive. Not all patients are eligible for government-funded treatment. Most patients cannot afford to pay themselves. Androgenic steroid is an alternative drug for the treatment of anaemia associated with uraemia. In addition, it has beneficial effects on nutrition.

Methods: New PD patients were recruited. They were informed of the adverse effects. Deca Durabolin was given intramuscularly every 1 to 2 weeks. Adverse reaction was documented on clinic visits. Hemoglobin level, serum albumin and liver enzymes values were collected. An increment of hemoglobin of 1 gm/dl over baseline was intepreted as positive response.

Results: 11 patients consented to the study. Male : female = 6 : 5. The mean age was 67.5 (range 41–80). 8 patients had ESRD due to unknown etiology. 1 had DM, 1 had IgA nephropathy and 1 had lupus nephritis. The baseline hemoglobin level and serum albumin level were 7.0 g/dl (3.7–9.5 g/dl) and 34.4 g/L (27–43 g/L) respectively. 10 patients were available for analysis at 6 and 12 months and 8 remained in the study at 18 months. 60%, 50% and 50% of patients had positive hemoglobin responses at month 6, 12 and 18 respectively. The mean serum albumin levels were 36.7, 35.6 and 35 g/L at month 6, 12 and 18 respectively. No hepatitis was observed. Hirsutism was reported in 2 female patients while acne was seen in 1 male and 1 female patient. No patient discontinued the injection. No injection pain was reported.

Conclusions: Deca Durabolin is effective in half of the patients over a period of 18 months. Adverse reaction was uncommon. All patients continue the treatment despite some reports of hirsutism and acne formation. Therefore we conclude that Deca Durabolin is both an effective and well-tolerated treatment for anaemia in PD patients.

W-PO40124

INTRAVENOUS ASCORBIC ACID IN HAEMODIALYSIS PATIENTS WITH FUNCTIONAL IRON DEFICIENCY

MASIH NAGHIBI[1], FATEMEH NAZEMIAN[2], ABBAS-ALI ZERAATI[2]
Nephrology Division, Department of Medicine, Imam Reza Hospital, Mashhad University of Medical Sciences[1], Mashhad University of Medical Sciences[2]

Introduction: Hemodialysis (HD) patients with functional iron deficiency (FID) often develop resistance to recombinant human erythropoietin (rHuEpo). In these patients, iron therapy may be a hazard, leading to iron overload and consequently to hemosiderosis. Recent studies suggest that intravenous ascorbic acid (IVAA) may circumvent rHuEpo resistance. The aim of our study was to show the effects of IVAA on FID and whether this results in a better correction of anemia in HD patients with iron overload.

Methods: Fifty HD patients with serum ferritin >500 μg/l and Hematocrit (Hct) < 30% were selected and randomly divided into two groups, 25 patients received 300 mg of IVAA (IVAA group) and 25 patients did not (control group) after each dialysis session. Hematocrit (Hct), transferrin saturation, ferritin, weekly recombinant human erythropoietin (rHuEPO) dosage and kt/v were measured at baseline and after 8 weeks of treatment.

Results: We observed a significant increase in Hematocrit and transferrin saturation (baselines vs 8 weeks, Hct 27.6% ± 2.27 vs 31.7% ± 4.18, TS% 33 ± 0.17 vs 42 ± 0.17, respectively p = 0.002 and p < 0.05) and a decrease in ferritin (from 1027 ± 566 to 732 ± 395 μg/L p = 0.02) level in IVAA compared with control group. The total weekly-required HuEpo dose also fell significantly in IVAA compared with control group (5440 ± 1588 vs 6770 ± 1336 p = 0.001).

Conclusion: IVAA may partially correct FID and consequently help rHuEpo hyporesponsive anemia.

W-PO40125

EVALUATION OF THE EFFECTS OF INTRAVENOUS CARNITINE ON HEMOGLOBINE AND HEMATOCRIT LEVEL IN HEMODIALYSIS PATIENTS

ESMAEEL ZAMZAMI[1]
Zamzami Esmaeel MD[1]

Objective: Evaluation of the effects of intravenous carnitine on hemoglobine and hematocrit level in hemodialysis patients.

Introduction: Anemia is one of the commonest complications of hemodialysis and is seen in 60–80% of hemodialysis patients. Recombinant erythropoietin is the best treatment of anemia but it is very expensive. Many studies have shown that carnitine cause improvement of anemia in this patients but there is not enough evidence for adminstration of carnitine for all hemodialyzed patients. Therfore, we designed this study to evaluate the effect of carnitine on hematocrit and hemoglobine level in hemodialyzed patients.

Method: During this double blind placebo controlled randomized clinical trial we chose patients who had been dialyzed at least 1 year and after rulling out of other cause of anemia, we divided patients in two groups. Case group received lgr camitine intravenously after each dialysis session and placebo group recieved distilled water as a placebo.

Also a questionnaire about nausia, vomiting, cramp, symptomatic hypotention for each patient is completed. Data were analyzed with spss 11/5 and paired T-Test used, for comparison of Hb and HCT between two groups.

Results: In this study there were 14 patients in carnitine group and 15 patients in placebo group. The mean of age and sex and the distribution of Hb and HCT was similar in both groups before intervention, but the mean of Hb and HCT after intervention in carnitine group significantly increased (P = 0/001, P = 0/0001).There was no statistical difference in cramp, nausia, vomiting and symptomatic hypotension.

Discussion and Conclusion: Finding of this study shows that intravenous carnitine increases Hb and HCT in hemodialysis patients and cause improvement of anemia.

W-PO40126

EFFECT OF PARATHYROIDECTOMY ON ANEMIA IN ESRD PATIENTS ON HEMODIALYSIS

SREEKRISHNA VENUTHURUPALLI[1], BALASUBRAMANIAN SUBBARAYAN[1], PRABHAKAR KOWDLE SATHYANARAYANA[1], WONG KOK SENG[1], NANDAKUMAR MOOPPIL[1]
National Kidney Foundation, Singapore[1]

Introduction: Elevated parathyroid hormone level is believed to contribute to anemia in patients on hemodialysis. Correction of this hyperparathyroid state either medically or surgically is expected to improve Hemoglobin. We retrospectively analyzed the data of patients who had parathyroidectomy for improvement in anemia.

Methods: Data of patients on hemodialysis with National Kidney Foundation, Singapore, who had surgical correction for hyperparathyroidism from 2001 to 2004 were included. Hemoglobin (Hb) was monitored every two weeks. Complete iron profile was obtained every 2 months including Serum iron, Ferritin and Transferrin Saturation (TS%). Parathyroid Hormone (iPTH) levels were monitored post operatively. Any change of Erythropoietin (EPO) dose was recorded. These patients were divided into 3 groups based on response of Hb at six months post operatively, GroupI: Non-responders, Group II: Hb increase <1 g/dl and Group III: Hb increase >/= 1 g/dl, compared to pre operative levels.

Results: 48 patients were included in the study consisting of 27 females and 21 male patients with mean age of 54.4 years. The Hb at 2 months after surgery was significantly lower (11.2 ± 1.6 Vs 10.7 ± 1.5 g/dl)(p = 0.000) reflecting the immediate effects from surgery. Subsequently Hb improved. However, rise of Hb at 4 and 6 months was not significant compared to the pre operative levels. There was no significant difference in EPO doses and no significant change in serum Iron, Ferritin and TS% levels at four and six months. Group II and III had significantly higher Pre operative iPTH levels compared to Group I (206 Vs 258 Vs 156 pmol/L respectively, p = 0.001).

Conclusion: Contrary to reports available in literature, we did not find significant improvement in Hemoglobin after parathyroidectomy. Pre operative iPTH levels positively predict the improvement in Hemoglobin at 6 months.

W-PO40127

LONG-TERM INTRAVENOUS CERA (CONTINUOUS ERYTHROPOIETIN RECEPTOR ACTIVATOR) MAINTAINS HEMOGLOBIN CONCENTRATIONS IN HEMODIALYSIS PATIENTS

ANATOLE BESARAB[1], ULRICH BEYER[2], FRANK C DOUGHERTY[2]
Henry Ford Hospital, Detroit[1], F. Hoffmann-La Roche[2]

Improving the fraction of patients maintained within guideline target Hb ranges is of paramount importance for the optimal management of anemia in patients with chronic kidney disease (CKD). Continuous Erythropoietin Receptor Activator (CERA), an innovative agent acting differently at the receptor level and with prolonged half-life, is currently in development to provide rapid, sustained and stable correction of anemia in CKD patients with extended dosing intervals. We conducted a multicenter, Phase II study of intravenous (IV) CERA in patients with chronic renal anemia on hemodialysis. Patients previously treated with IV epoetin received IV CERA administered once weekly (1x/wk) or once every 2 weeks (1x/2 wks). Patients were followed for 19 weeks in total. Thereafter, 53 patients entered a 12-month extension period with the aim of maintaining Hb levels at 11–12 g/dL. Data from the core period have been reported previously (Besarab et al. EDTA 2004). Here, we report on the findings of the 12-month extension period during which patients were dosed at 1x/wk or 1x/2 wk intervals.

Over the course of the 12-month extension period, mean Hb levels for the two groups, measured on a monthly basis, ranged from 11.03 to 12.02 g/dL. Mean Hb levels over time were 11.36 g/dL (95% CI: 10.98, 11.73) and 11.51 g/dL (95% CI: 11.31, 11.71) in the 1x/wk and 1x/2 wk administration cohorts, respectively. Overall, CERA was generally well tolerated and the frequency of adverse events appeared to be characteristic of this patient population.

These data suggest that IV CERA may achieve sustained and stable control of Hb within guideline ranges and confirms our preliminary findings in hemodialysis patients. Stable Hb levels achieved with extended dosing intervals may optimize personnel time in dialysis units and could potentially improve outcomes for patients.

W-PO40128

THE EFFECT OF CONTINUOUS QUALITY IMPROVEMENT (CQI) ON ANEMIA MANAGEMENT IN HEMODIALYSIS PATIENTS

MIN CHEN[1], JINHUA DENG[1], FUDE ZHOU[1], MEI WANG[1]

Renal Division and Institute of Nephrology, Peking University First Hospital, Beijing P.R. China[1]

Background: Anemia is common in hemodialysis patients, which can diminish the quality of live substantially. Continuous quality improvement (CQI) programs have been instituted in many health care organizations over the last decade. Improvement of anemia management is possible with the implementation of CQI.

Objective: To improve anemia management in chronic hemodialysis patients using CQI.

Method: Ninety hemodialysis patients in our single center were enrolled in the study, all of whom were dialysed more than 3 months. The patients were followed from the Jan 1, 2004 to Jan 1, 2005. The CQI team-oriented approach was driven by a four-step process called PDCA cycle-plan: Plan, Do, Check and Act. Hemoglobin was measured once a month. Serum iron, ferrtin and transferritin saturation was monitored every three to four months.

Results: After the CQI program, the hemoglobin of the patients increased from 101.2 ± 18.05 g/L to 109.8 ± 16.67 g/L ($P < 0.01$). The proportion of patients with Hb ≥ 110 g/L increased from 42.2% to 52.2%. The proportion of patients with absolute iron deficiency was dropped from 21.1% (4/19) to 8.9%(8/90), and the proportion of patients with functional iron deficiency was dropped from 57.8%(11/19) to 22.2%(20/90). The weekly dose of rhEPO did not change significantly. Clinically, the improvement of anemia was achieved through the following ways: (1) Improve the patients' compliance of taking iron by repeated education. (2) For the 15 patients whose oral way of iron supplementation could not meet the need, switched from oral to intravenous way. (3) Reduce the blood loss from coagulation of dialyzer by adjust the dose of heparin and the blood flow rate during hemodialysis.

Conclusion: Continuous quality improvement program is a useful method in management of anemia in hemodialysis patients.

W-PO40129

INTERACTION OF MALNUTRITION WITH EFFICIENCY OF ANEMIA TREATMENT IN HD PATIENTS: THE OPTIMAL TREATMENT OF RENAL ANEMIA INITIATIVE (OPTA)

MARTIN K. KUHLMANN[1], WALTER H. HORL[2]

Renal Research Institute, New York[1], University of Vienna, Austria[2]

International practice guidelines define Hgb levels between 11.0–12.0 g/dl as treatment target for renal anemia. However, data from the Dialysis Outcome and Practice Pattern Study (DOPPS) and the European Survey on Anemia Management (ESAM) indicate that Hgb levels <11.0 g/dl are found in 47% of patients and in 40–50% of these, Hgb levels are <10 g/dl despite EPO availability. OPTA aims to develop strategies for optimal anemia treatment with highest efficiency. Efficiency of EPO therapy is influenced by various factors, such as dialysis dose, iron status, inflammation and nutritional status. Malnutrition (MN) is common and related to chronic inflammation, morbidity and mortality. Most biochemical indicators of nutritional status (e.g. albumin), are negatively associated with inflammation. In the absence of inflammation serum cholesterol <150 mg/dl and low prealbumin levels appear to be good indicators of MN.

Malnutrition may influence EPO responsiveness independent of inflammation by low intake of vitamins B, C and E, impaired total antioxidant capacity, decreased dietary iron supply and increased catabolism. Anorexia itself is associated with EPO hyporesponsiveness, a 4-fold increase in mortality, greater hospitalization rates, and poor quality of life. Better nutritional status is associated with lower EPO dose and some studies indicate that improving nutritional status and reversing anemia both independently improve EPO response. A postulated mechanism is that EPO may exhibit anabolic effects, with better utilization of ingested protein.

Conclusion: Nutritional status is an important factor influencing EPO response, both, dependent and independent of inflammation. The OPTA working group recommends that HD patients unresponsive to EPO therapy, with decreasing BMI and total cholesterol <150 mg/dl be considered for nutritional support. Improvement of nutritional status may eventually have positive economic implications on anemia management.

W-PO40130

SAFETY AND TOLERABILITY PROFILE OF CONTINUOUS ERYTHROPOIETIN RECEPTOR ACTIVATOR (CERA) WITH EXTENDED DOSING INTERVALS IN PATIENTS WITH CHRONIC KIDNEY DISEASE ON DIALYSIS

FRANK C DOUGHERTY[1], ULRICH BEYER[1]

F. Hoffmann-La Roche[1]

Continuous Erythropoietin Receptor Activator (CERA), an innovative erythropoiesis-stimulating agent acting differently at the receptor level and with prolonged half-life, is currently in development to provide sustained and stable correction of anemia with extended dosing intervals in patients with chronic kidney disease (CKD).

Two multicenter, Phase II studies of CERA were conducted in dialysis patients. Patients previously treated with epoetin were treated with intravenous (IV) CERA (1x/wk or 1x/2 wk) or subcutaneous (SC) CERA (1x/wk, 1x/3 wk or 1x/4 wk*) for a total of 19 weeks (*21 weeks). Thereafter, patients entered 12-month extension periods with the aim of maintaining Hb levels at 11–12 g/dL. Here, we report on the combined safety and tolerability of IV/SC CERA during these extension periods in 109 patients.

In these two studies patient characteristics at baseline were as follows: 63% of patients were male, 86% were aged ≥45 years, 61% were Caucasian, and 36% were diabetic. The mean duration on dialysis was 3.68 ± 4.14 years.

CERA administered SC and IV in dialysis patients was generally well tolerated. The most common adverse events (AE) were hypotension, muscle cramp, hypertension, headache and nasopharyngitis, which accounted for 8.02%, 4.39%, 3.05%, 2.86% and 2.10% of all AE episodes, respectively. The most common serious adverse events were hypotension (5 events), myocardial infarction (5 events), cellulitis (4 events) and pancreatitis (4 events).

In conclusion, these results indicate that CERA administered with extended dosing intervals (up to 1x/4 wk) was generally well tolerated in dialysis patients. In addition, the frequency of the adverse events reported appears to be characteristic of this patient population.

W-PO40131

NO CHANGES IN BLOOD PRESSURE IN DIALYSIS PATIENTS AFTER 12 MONTHS OF TREATMENT WITH IV/SC CERA (CONTINUOUS ERYTHROPOIETIN RECEPTOR ACTIVATOR)

FRANK C DOUGHERTY[1], ULRICH BEYER[1]

F. Hoffmann-La Roche[1]

Continuous Erythropoietin Receptor Activator (CERA), an innovative erythropoiesis-stimulating agent acting differently at the receptor level and with prolonged half-life, is currently in development to provide sustained, stable correction of anemia with extended dosing intervals in patients with chronic kidney disease (CKD).

We conducted two multicenter, phase II studies of CERA in dialysis patients who were previously treated with epoetin, one study investigating intravenous (IV) administration (1x/wk or 1x/2 wk), the other subcutaneous (SC) administration (1x/wk, 1x/3 wk or 1x/4 wk*). Patients were followed for a total of 19 weeks (*21 weeks). Thereafter, patients entered 12-month extension periods with the aim of maintaining Hb levels at 11–12 g/dL. Here we report on blood pressure parameters before and after dialysis at the start of the extension period (baseline) and after 12-months of treatment with CERA (study end) in 112 patients.

Patient characteristics at baseline were as follows: mean age 60 ± 13 years, mean systolic blood pressure (SBP) 146 ± 29 mm Hg, mean diastolic blood pressure (DBP) 78 ± 17 mm Hg, mean body mass index (BMI) 26.8 ± 5. The main causes of renal disease were hypertension (30%), diabetes (28%) and glomerulonephritis (13%).

Regardless of the route of administration, no relevant differences in blood pressure parameters before dialysis sessions were noted between baseline and study end (Table). Similar findings were noted for blood pressure parameters after dialysis sessions. Overall, CERA was generally well tolerated and the frequency of adverse events appeared to be characteristic of this population.

In conclusion, no relevant changes in blood pressure parameters were observed after 12 months of treatment with CERA administered at extended dosing intervals (up to 1x/4 wk) indicating that in these Phase II studies blood pressure was adequately controlled in dialysis patients treated with IV or SC CERA.

	Blood pressure before dialysis sessions (mean, 95% CI) (IV/SC studies combined, n = 112)			
	SBP	DBP	PP	MAP
Baseline (start of extension period)	146 (140, 151)	78 (75, 81)	67 (63, 71)	100 (97, 104)
Study end	133 (121, 145)	77 (71, 83)	58 (50, 66)	96 (89, 104)

SBP; systolic blood pressure; DBP diastolic blood pressure; PP: pulse pressure; MAP: mean arterial pressure

W-PO40132

IRON STATUS AND IRON SUPPLEMENTATION IN HEMODIALYSIS PATIENTS TREATED BY EPOETIN BETA: FRENCH OBSERVATIONAL STUDY

MICHELE KESSLER[1], ERIC CANIVET[2], LAURENT YVER[3], PIERRE BATAILLE[4], GEORGES BRILLET[5], AYMERIC DE CHASTEIGNER[6], BÉNÉDICTE COMMENGES[6], PAUL LANDAIS[7]

Department of Nephrology, Nancy, France[1], ARPDD, Reims, France[2], Department of Nephrology, Angoulême, France[3], Department of Nephrology, Boulogne-sur-mer, France[4], Nephrology Center, Châteauroux, France[5], Roche, Neuilly-sur-Seine, France[6], Department of Biostatistic and Medical Informatics, Necker, Paris, France[7]

Background: Iron deficiency limits the efficacy of epoetin therapy in hemodialysis patients. The iron status and the patient iron supplementation have been evaluated in routine nephrology in the DiaNE study about the management of anemia in French hemodialysis centers.

Protocol and patients: This analysis of baseline data was conducted on 1233 hemodialysis patients (mean age, 66 y; men, 55%) treated by epoetin beta (EB) and followed up for one year. The median duration of treatment by any recombinant human erythropoietin was 3 y and 1.4 y for EB.

Results: Sixty five percent of patients received epoetin beta by subcutaneous (SC) route and 35% by intraveinous (IV) route. Weekly dosages of EB were significantly lower ($p < 0.0001$) in patients with SC as compared to patients with IV (median dosage: 6000 *vs* 8000 UI).

IV iron supplementation was more frequently used in patients treated by SC as compared to IV (64 *vs* 55%; $p < 0.002$); mean doses of IV iron supplementation were 92 ± 62 and 83 ± 53 mg/week, respectively. Iron status was similar in the 2 groups: serum iron 59.8 ± 47.7 and 69.3 ± 115.7 µg/100 mL, serum ferritin 346 ± 234 and 360 ± 239 µg/L, transferrin saturation coefficient (TSAT) 26.7 ± 12.1 and 27.2 ± 11.8%, respectively.

A multivariate analysis found TSAT >20% ($p = 0.0014$), serum albumin >30 g/L ($p = 0.02$), C-reactive protein <10 mg/L ($p = 0.0034$), presence of glomerulopathy ($p = 0.0065$) and EB dosage <150 UI/kg/week ($p = 0.0002$) significant indicator of Hb target achievement (Hb > 11 g/dL).

Conclusion: No difference exists beetween SC and IV epoetin in the iron status but IV iron supplementation is more frequently used in patients treated by SC. Transferrin saturation coefficient appears to be the best iron parameter to predict success of epoetin beta treatment.

W-PO40133

SENSITIVITY TO EPOETIN (ESA) IS AN INDICATOR OF SURVIVAL IN DIALYSIS PATIENTS

ALISON ROCHE[1], ANURADHA JAYAPRAKASAM[1], IAIN MACDOUGALL[1], LARISSA MILES[2], MARIA LUISA AGUERA MORALES[3]

King's College Hospital, London[1], University of Newcastle, Australia[2], Renal Unit, Hospital Reina Sofia, Cordoba, Spain[3]

The aim of this retrospective cohort study sought to investigate the interrelationship between Hb level, Epoetin (ESA) dose, and survival in patients with ESRD.

564 patients were identified as receiving ESA therapy in August 2002. The exclusion criteria of CKD patients not on RRT (n = 259), transplantation during study period, patients on ESA < 90 days; death within 90 days of censored date (n = 86), left 219 patients within the analysis. The remaining patients were divided into 4 groups based on their sensitivity to epoetin therapy.

Group	Haemoglobin (g/dl)	EPO dose (iu/kg/week)
Group A	>11	<150
Group B	>11	>150
Group C	<11	<150
Group D	<11	>150

Their survival over the subsequent 2 years was evaluated using both quantitative (Students t test) and qualitative (Kaplan-Meier plots and log rank) analyses.

The survival of the 4 groups of patients (most sensitive to least sensitive) during the 2-year study period was: Group A, 61.7%; Group B, 55.4%; Group C, 50%; Group D, 30.8% (log rank test $p < 0.005$). Thus, the epoetin-resistant patients in group D had by far the worst survival ($p < 0.005$ versus Group A, $p < 0.01$ versus Group B, and $p = 0.08$ versus Group C); there was no significant difference ($p = 0.63$) between survival in Groups B and C. Survival in Group B patients was worse than in Group A patients despite similar Hb levels (>11 g/dl), and patients receiving high (>150 U/kg/week) epoetin doses (Groups B and D combined) had a worse survival compared to those receiving low (<150 U/kg/week) epoetin doses (Groups A and C combined) ($p < 0.05$).

The findings from this study confirm that anaemia is an important indicator of survival in dialysis patients, but suggest in addition that sensitivity to epoetin is likewise a significant marker of survival. Nephrologists should perhaps pay more attention to the investigation of causes of epoetin resistance rather than simply escalating the doses of epoetin.

W-PO40134

INTRAVENOUS ASCORBIC ACID IN COMPARISON TO INTRAVENOUS IRON IN ERYTHROPOIETIN RESISTANT ANEMIA WITH IRON OVERLOAD IN HEMODIALYSIS PATIENTS

MONIR S. HAKEMI[1], MOHAMMAD REZA GANJI[2], IRAJ NAJAFI[2], MOHSEN SHEKARCHI[2]

Academy of Medical Sciences[1], Department of Nephrology, Dr. Shariati Hospital, TUMS[2]

Functional iron deficiency may develop and cause erythropoietin (EPO) resistant anemia in hemodialysis (HD) patients. It has been suggested that EPO resistant anemia associated with iron overload can be overcome by ascorbic acid administration. It is controversial whether iron therapy will improve EPO resistant anemia or not.

A randomized controlled trial was conducted to study the effect of intravenous ascorbic acid (IVAA) versus intravenous iron (IVFE) in the treatment of EPO resistant anemia with iron overload in HD patients.

Sixty-three EPO resistant HD patients with hemoglobin (HB) < 10 g/dl, transferrin saturation (TS %) < 30% and serum ferritin levels >500µg/l were randomly divided into IVAA and IVFE groups. In IVAA group, intravenous ascorbic acid (500 mg) two times a week for 8 weeks was prescribed. In IVFE group, ferric saccharate (100 mg) was administered post dialysis on 5 consecutive dialysis sessions. Hb, hematocrit (Hct), TS% and serum ferritin levels were checked before and 12 weeks after the study. Serum iPTH and aluminum levels were checked at the start of study and didn't show significant difference between two groups. In IVFE group, mean Hb, Hct and TS% didn't change significantly after the study. In contrast, in IVAA group, mean Hb level increased significantly from 9.2 ± 0.2 to 10.2 ± 0.2 ($P = 0.000$) and also TS% increased from 21.4 ± 1.1 to 22.6 ± 1 ($P = 0.04$) In contrast to previous reports, we observed that also in IVAA group, serum ferritin increased but we couldn't find any explanation for the increased serum ferritin levels. Our study shows that short- term administration of ascorbic acid is effective as an adjuvant therapy in EPO resistant anemia with iron overload in HD patients.

W-PO40135

EFFICACY OF IV OR SC DARBEPOETIN ALFA IN HEMODIALYSIS PATIENTS

JUERGEN BOMMER[1], H.-G. ASMUS[2], G. BOMMER[3], M. WENNING[4]
Medical University Heidelberg[1], KFH Berlin Sonnenallee[2], Heimdialyse Heidelberg[3], Augusta Krankenanstalten, Bochum[4]

The equipotent iv rHu-EPO dose is about 25–30% higher than the sc dose (Kaufmann et al., 2000). We analyzed the efficacy of sc or iv Darbepoetin alfa in a prospective, randomized multicenter study.
Material and Methods: In an ongoing study 102 Pats, mean age 62.0 ± 14.3 years, duration on Dialysis 5.5 ± 5.8 yrs, continuously under sc Darbepoetin alfa therapy with stable dose and haemoglobin levels during the previous 8 weeks, were randomized to iv or continuous sc Darbepoetin alfa therapy. 12 patients were excluded for violation of the protocol or transplantation. Causes for ESRD and hypertension were comparable in both groups. Baseline serum ferritin concentration was 603 ± 259 ng/ml (median 581) and comparable in both groups. EC approval and informed consent of pats were obtained.
Results: Means and medians of haemoglobin, weekly Darbepoetin alfa (Darb) dose and serum ferritin remained similar in patients who switched to iv therapy compared with patients under continuous sc therapy.

	basal	24 weeks	48 weeks
IV Therapy			
N =	43	36	35
Hb (g/dl)	34.5*	35.8*	34.9*
Darb ug/wk	31.2 ± 17.4**	33.7 ± 21**	31.7 ± 21.4**
SC Therapy			
N =	49	48	38
Hb (g/dl)	35.5*	36.1*	34.8*
Darb ug/wk	26.3 ± 17.1**	25.6 ± 18.6**	30.5 ± 20.9**

*: median, **: mean ± SD

Conclusions: The data suggest that the efficacy iv and sc darbepoetin alfa is comparable in contrast to iv or sc rHu-EPO.

W-PO40136

THE EFFECT OF GLYCAEMIC CONTROL ON THE RESPONSIVENESS OF EPOIETIN BETA (EB) AND DARBEPOIETIN ALPHA (DA) ON DIABETIC PERITONEAL DIALYSIS (PD) PATIENTS: A PROSPECTIVE STUDY

ANTHONY YINKEET CHAN[1], ELEANOR MALONEY[1], ALISTAIR CHESSER[1], RAJ THURAISINGHAM[1], MARTIN J RAFTERY[1], STANLEY FAN[1], MUHAMMAD MAGDI YAQOOB[1]
Barts and The London NHS Trust[1]

Diabetic patients with renal failure develop anaemia early in the course of renal disease. The relationship between erythropoietin (EPO) responsiveness and glycaemic control is unknown. We examined the effect of glycaemic control on the efficacy of EPO therapy in diabetic patients on PD. Seventy iron replete (ferritin > 100 μmol/l) patients (27F) on PD for more than 3 months were studied for a year. Patients freely chose to commence either subcutaneous EB (started at 2000iu per week) or DA (10 μgm/week). Monthly dose adjustments were carried out as per local protocol (target haemoglobin (Hb) 11–13.5 gm/dl). Patients were divided into three groups based on time averaged HbA1c (<7.5–25 patients, 7.5–8.5 – 18 patients and >8.5 – 27 patients). Patients and blood results were reviewed every 2 months.
The groups were similar in age, gender, duration of PD and proportion requiring insulin. They were also comparable for serial PTH, Ca, PO4, ALP, CRP. The time averaged Hb (gm/dl) and EPO dosage (IU/ Kg/week using a 200 to 1 conversion factor between EB and DA) in the three groups were 11.2 ± 1.4, 12.2 ± 1.7, 11.8 ± 1.3 and 104 ± 78, 66 ± 48, 88 ± 59 respectively (p = ns). Only 23 patients chose DA: most patients preferred EB, either because they preferred Recopen (only available with EB) or because of less pain at the injection site. The efficacy of DA and EB was similar with respect to time averaged Hb of 12.0 ± 1.5 and 11.4 ± 1.3 at doses of 0.46 + 0.35 μg/kg.wk and 84.5 + 62.1 IU/kg/wk respectively, equating to a conversion factor of 1 to 184.
We conclude that glycaemic control has no effect on EPO responsiveness in PD patients. The bioequivalence between EB and DA achieved was close to current recommendations.

W-PO40137

IMPACT OF INFLAMMATION/INFECTION ON ANEMIA THERAPY IN HAEMODIALYSIS PATIENTS: THE OPTIMAL TREATMENT OF RENAL ANEMIA (OPTA) INITIATIVE

CHRISTOPH WANNER[1], DONALD RICHARDSON[2], PETER STENVINKEL[3]
Department of Nephrology, University Hospital Würzburg Germany[1], Nephrology, District Hospital, York, United Kingdom[2], Nephrology, Huddinge University Hospital, Stockholm, Sweden[3]

A variety of factors have impact on epoetin therapy such as dialysis dose, iron status, blood loss and nutritional status, but infection/inflammation is the major treatment influencing factor in the management of anemia. Infection/inflammation induces resistance to epoetin leading to hyporesponsiveness or escalating epoetin doses. C-reactive protein (CRP) is the most commonly used biomarker and indicator of an activated acute phase response. CRP levels >10 mg/l already lead to a 34% higher epoetin dose as compared to CRP levels <10 mg/L. This projects aims to develop models and recommendations to overcome epoetin resistance by dissecting inflammation/infection through regular high sensitive CRP determinations in given dialysis populations.
This study included 720 patients, of whom 300 incident dialysis patients, from 3 renal units in Sweden, UK and Germany which served as a data base to develop a model for surveillance of the inflammatory status in dialysis patients. Unselected dialysis patients, treated at the centers at a given day, were included in the present study. High sensitive CRP provided a typical distribution curve in a hemodialysis unit. In following steps CRP distribution curves from national-regional data are compared with the developed model. Hypothetically, the distribution curves of CRP in a hemodialysis unit with poor water quality or a Unit with access problems should reveal deviating curves. This approach may serve as a first indicator/benchmark of the inflammatory status in a given dialysis unit.
These data suggest that (1) a classification of CRP ranges can be used in a diagnostic algorithm, (2) a clinical and a diagnostic procedure can be established on a unit and on an individual patient level, (3) typical inflammatory conditions and infectious diseases can be identified. This approach may contribute towards quality improvement in renal care.

W-PO40138

AUDIT OF TRANSFUSION IN CHRONIC RENAL FAILURE PATIENTS UNDERGOING TRANSPLANTATION: CHANGES OVER A DECADE

PRITI ELHENCE[1], PRASHANT PANDEY[1], RAJENDRA K CHAUDHARY[1], RAJ KUMAR SHARMA[3]
Dept of Transfusion Medicine, SGPGIMS India[1]; Dept Of Transfusion Medicine[2]; Dept Of Nephrology[3]

Introduction: Patients undergoing renal transplantation require blood component transfusion during pre, perop and postop period. Transfusion policies for these patients have undergone several changes over the last few decades. Beneficial and adverse effects of red cell transfusion on graft and patient outcome has been a subject of debate. Shorter waiting period, availability of erythropoietin, better immunosuppresives and better surgical techniques have also said to affect the transfusion requirements of the patients. Hence a reterospective study was conducted to find out the change in transfusion practices during pre, per and post transplant period at a interval of a decade.
Material and Methods: A restrictive transfusion policy is being followed. Transfusion record and patient demographic data for 124 patients undergoing live related renal transplantation during year 2004 was retrieved from records on Hospital information System software. 14 patients were excluded. Relevant patient data was taken from the files. Data was compared with similar analysis done in 1994 on 54 live related renal transplant patients.
Results: Despite restrictive transfusion policy the blood usage during the pre transplant period has increased. During 1994, 72.2% of patients received 1–5 units and 22.2% received 6–10 units whereas in 2004 only 48.2% received 1–5 units and. 46.2% received 6–10 units. During both the periods group O persons

required significantly greater number of transfusions. Red cell usage during transplant surgery has decreased significantly (Mean Tx 2.1 units in 2004 vs 4.08 in 1994) although the usage of Fresh Frozen plasma and platelets has increased. Data was analysed for factors having potential effect on transfusion practice.
Conclusion: Audit of transfusion practice helped in analysing conformance to the guidelines and gave the real picture.

W-PO40139

SERUM SOLUBLE TRANSFERRIN RECEPTOR IN DIABETIC AND NON DIABETIC UREMICS

IRENA PIETRZAK[1], DOROTA FORMANOWICZ[1]
Department of Nephrology, Transplantology and Internal Medicine, University of Medical Sciences in Poznan, Poland[1]

Serum ferritin (SF) and transferrin (Tf) can be influenced by inflammatory processes. The soluble transferrin receptor (sTfR), marginally influenced by inflammation, has been proposed an indicator of Fe stores. The aim of study was to compare sTfR concentration and correlations with Tf, Fe concentration, Fe and EPO dose in diabetic (DN) and nondiabetic (NDN) patients. Fifty ESRD patients (aged 55.6 ± 14 years) were divided into 2 groups: DN ($n = 17$) and NDN ($n = 33$). All patients received EPO and intravenous Fe. Fifty healthy volunteers (HV) aged 42 ± 5 formed a reference group. Serum samples were drawn at the start of HD. STFR (mg/l) was determined by immunonephelometry, Tf (mg/l) by immunoelectrophoresis, serum EPO by ELISA, Hb, UIBC, Fe (mmol/l) by colorimetric and SF by chemiluminescence method. sTfR concentration did not differ significantly between DN $1.27 \pm .15$ and NDN 1.15 ± 1.07 and HV 1.26 ± 0.5. Tf in DN 1423.4 ± 350.5 and in NDN 1427.9 ± 518 significantly lower than in HV 3525.1 ± 757.1. sTfR did not correlate with Tf. Fe concentrations in DN 14.43 ± 7.92 and NDN 14.385 ± 7.409 were lower than in HV 18.73 ± 9.1 ($p = 0.08$, $p = 0.025$ respectively). No correlation was found between sTfR and serum Fe, dose of Fe and EPO dose. sTfR was correlated with UIBC in DN ($p = 0.019$). In NDN sTfR correlated with BMI, RBC and TIBC ($p = 0.045$, $p = 0.03$, $p = 0.02$ respectively), contrarily to DN. Correlation was found between R/F ratio (sTfR/log SF) and serum Fe and dose of Fe in NDN group ($p = 0.0016$, $p = 0.0001$) respectively. There were no correlations between R/F ratio and EPO dose in both groups. In summary the R/F ratio proved to be useful in estimating body iron stores in haemodialysed patients. The presence of diabetes mellitus did not change significantly the values of sTfR and serum Fe concentration in uremics.

W-PO40140

THE ROLE OF FERRITIN AS AN INFLAMMATORY RESPONSE MARKER IN THE EVALUATION OF ANEMIA IN HEMODIALYSIS PATIENTS

BENEDITO JORGE PEREIRA[1], ADRIANA PEIXOTO GELMETTI[1], EZEQUIEL RIOS ARIZA[1], ADERBAL ÂNGELO NASTRI[1]
Nephrology, Brigadeiro Hospital, Brazil[1]

Ferritin is a non-specific acute phase protein, occurring in high serum levels during inflammation or uremia. The presence of unacceptable anemia (hemoglobin < 11 g/dl; hematocrit < 33%, DOQI), in uremic patients making use of erythropoietin (EPO) with adequate iron storage levels, may signal systemic inflammatory process.
Objectives: To evaluate the prevalence of unacceptable anemia in asymptomatic patients undergoing hemodyalisis; to evaluate the possible risk factors for unacceptable anemia and their importance with respect to ferritin serum levels.
Methods: 29 hemodyalisis patients were evaluated *regarding* serum levels of hemoglobin, transferrin saturation (Tsat), ferritin, parathyroid hormone (PTH), calcium-phosphorus product, and doses of EPO and IV iron. For each patient three results from the last five months were selected at random, and subsequently divided into two groups: patients with unacceptable anemia (group I) and with acceptable anemia (group II). Data underwent statistical analysis (non paired t-test) and results were presented using mean, standard deviation and percentage.
Results: 13 patients had unacceptable anemia (group I-44.8%) and 16 had acceptable anemia (group II-55.2%). Mean age was 50.5 ± 14 years (group I) and 47.3 ± 11.6 years for (group II), NS. *Diabetes mellitus* occurred in 38.4% (group I) 18.7% (group II), NS; hemoglobin (g/dl) $= 9.8 \pm 0.74$ (group 1) 11.8 ± 0.7 (group II), $p < 0.05$; Tsat (%) $= 34.5 \pm 13.8$ (group I) 43.0 ± 39.7 (group II), NS; ferritin (ng/ml) $= 527.7 \pm 123.8$ (group I) and 1154.0 ± 720.6 (group II), $p < 0.05$; Ca × P product $= 51.3 \pm 10.5$ (group I) and 57.7 ± 11.9 (group II), NS; PTH (pg/ml) $= 728.4 \pm 524.2$ (group I) and 457.8 ± 438.1 (group II). EPO doses UI were $10.615.4 \pm 3.000$ (group I) and 6.000 ± 3.651 (group II); IV iron doses (mg) were 170 ± 70 (group I) 150 ± 90 (group II).
Conclusions: Unacceptable anemia was found despite adequate iron storage levels and EPO doses being higher in this group. Higher ferritin levels in group II (acceptable anemia) suggest higher storage pools, with no correlation with anemia or inflammatory response in this group.

W-PO40141

RENAL ANEMIA IS NOT NORMOCYTIC NORMOCHROMIC BUT CHARACTERIZED BY MACROCYTIC HYPOCHROMIC SHIFT OF ERYTHROCYTE INDEX (EI)

MANABU OGURA[1], YASUHIRO ANDO[1], ATSUSHI KOTODA[1], HIDEAKI TAKAHASHI[1], NAOKI MURAYAMA[2], YASUSHI ASANO[1], EIJI KUSANO[1]
Nephrology/Jichi Medical School/Japan[1]*; Murayama Clinic/Japan*[2]

Renal anemia has been categorized as normocytic normochromic anemia unless deficiency of iron, folate or vitamin B12 is accompanied. However, in hemodialysis (HD) patients, increased MCV (mean corpuscular volume) and decreased MCHC (mean corpuscular hemoglobin concentration) are frequently found. We thus re-examined the characteristics of EI in chronic renal failure (CRF).
In healthy control (CONT, $n = 647$, mean serum Cr 0.7 ± 0.1 mg/dl), chronic kidney disease (CKD; serum Cr < 2, $n = 82$, mean serum Cr 0.9 ± 0.4), conservative period CRF (C-CRF; serum Cr > 2, $n = 19$, mean serum Cr 4.5 ± 2.3), HD group ($n = 190$, mean serum Cr 11.6 ± 2.9), EI were compared the factors associated with the changes in EI were analyzed.
The hemoglobin (g/dl), MCV (fl) and MCHC (%) were 13.6 ± 1.4, 94.0 ± 5.1, 32.6 ± 1.0 in CONT, 13.4 ± 1.9, 93.0 ± 5.0, 33.6 ± 0.7 in CKD, 9.6 ± 2.6, 98.1 ± 6.9, 32.9 ± 0.8 in C-CRF, 10.1 ± 1.1, 101.3 ± 6.6, 31.3 ± 1.3 in HD group, respectively. The differences in MCV and MCHC between CKD and C-CRF were significant (Mann-Whitney's U-test). In CKD and C-CRF ($n = 101$), MCV positively correlated with serum Cr ($p = 0.0001$; Spearman's rank correlation). In a case-series observation, these changes were noted even before anemia became apparent. The macrocytic and hypochromic transformation was more prominent in HD patients. Especially, cases with MCV > 100 fl and MCHC < 31% were found only in HD group (27% of HD group). Serum ferritin level and the use of recombinant erythropoietin were not related to these changes in EI. On the other hand serum Cl positively correlated with MCV both in C-CRF and HD patients.
In conclusion, MCV increases and MCHC decreases along with the progression of CRF. Ranal anemia, especially in HD patients, is uniquely characterized by macrocytic hypochromic transformation of erythrocyte.

W-PO40142

HEPCIDIN: A LINK BETWEEN ANEMIA, INFLAMMATION AND LIVER FUNCTION IN HEMODIALYZED PATIENTS

JOLANTA MALYSZKO[1], JACEK S MALYSZKO[2], TOMASZ HRYSZKO[2], MICHAL MYSLIWIEC[1]
Nephrology and Transplantology, Medical University, Bialystok, Poland[1]

Background: Hepcidin is a small defensin-like peptide whose production by hepatocytes is modulated in response to anemia, hypoxia or inflammation. Both anemia of renal disease and anemia of chronic inflammation results from an inappropriate production of erythropoietin, a blunted bone marrow response to erythropoietin due to various cytokines, an excessive blood loss, a reduced erythrocytes life span and reduced iron availability. Upregulation of acute-phase proteins is present in the majority of dialyzed patients and with chronic renal failure, thus an inappropriate hepcidin expression appears conceivable in these populations. Hepcidin could also act as an indicator of functional iron deficiency in these patients.
Aim: Hepcidin correlations with markers of iron status, erythropoietin therapy and markers of inflammation in 97 hemodialyzed patients and in the healthy volunteers.
Methods: Iron status (serum iron, total iron binding capacity-TIBC, ferritin, total saturation of transferin-TSAT), complete blood count, creatinine, albumin, serum lipids were assessed using standard laboratory method. Hepcidin and high sensitivity CRP were estimated using commercially available kits.
Results: Serum iron, TIBC, TSAT, erythrocyte count, Hb, Ht platelet count, albumin, cholesterol were lower in hemodialyzed patients when compared with the control group. Ferritin and hepcidin were significantly higher in hemodia-

lyzed patients relative to healthy volunteers. Hepcidin correlated positively with triglycerides, aspartate aminotransferase, lymphocyte count, ferritin and erythropoietin dose and negatively with erythrocyte count, Hb and Ht in hemodialyzed patients. Hepcidin did not correlate with CRP or albumin, considered as markers of inflammation. In multiple regression analysis serum triglycerides were the only predictor of hepcidin. In the healthy volunteers hepcidin was not related to any studied parameters.
Conclusions: Elevated hepcidin levels in hemodialyzed patients may be due to functional iron deficiency and anemia, but not to inflammation. Liver plays an important role in the synthesis of hepcidin.

W-PO40143

HEPCIDIN, A KEY MEDIATOR OF ANEMIA AND A MARKER OF FUNCTIONAL IRON DEFICIENCY IN INFLAMED DIALYZED PATIENTS (PTS)

GIOVANNI PERTOSA[1], SIMONA SIMONE[1], DANIELA MARRONE[1], ROBERTO CORCIULO[1], PAOLA PONTRELLI[1], SILVIA PORRECA[1], FRANCESCO P SCHENA[1], GIUSEPPE GRANDALIANO[1]
DETO, University of Bari, Italy[1]

A state of chronic microinflammation and a functional iron deficiency is frequently associated to anemia in dialyzed pts. Hepcidin, an antimicrobial peptide, is up-regulated during the acute phase response and might play an important role in iron homeostasis. We, therefore, addressed the question whether an inappropriate hepcidin expression may correlate with an altered iron metabolism and erythropoietin (EPO)-hyporesponsiveness in dialyzed pts. The relationship between serum hepcidin levels (kit ELISA), high sensitive C-reactive protein (hsCRP), some anemia parameters (Hb, iron and ferritin concentration) and weekly EPO dose was investigated in 176 uremic pts treated with hemodialysis (HD) (99 pts, male 66 female 33; mean age 62.7 years; dialytic age 40.3 months) or peritoneal dialysis (PD) (77 pts, male 47 female 30; mean age 57.2 years; dialytic age 31.1 months).
Significantly ($p < 0.0001$) higher serum levels of hepcidin were found in HD pts (174.1 ± 65.8) as compared to PD pts (98.0 ± 30.0) and normals (124.0 ± 13.5 mg/ml). Similarly, hsCRP were significantly ($p < 0.0001$) increased in HD (1.07 ± 0.8) compared to PD pts (0.5 ± 0.2 mg/l). Despite similar Hb levels (HD: 11.2 ± 1.2; PD: 11.1 ± 1.5 g/dl), ferritin concentrations were more elevated in HD (401.3 ± 274.0) than in PD pts (166.9 ± 162.0, $p < 0.0001$), whereas serum iron did not differ in the two groups (HD: 57.4 ± 20.0; PD:63.0 ± 21.0 mg/dl). Finally, weekly EPO requirements were significantly ($p < 0.001$) greater in HD (10.560 ± 7570 UI) than in PD pts (4430 ± 2616 UI). In the univariate analysis, serum hepcidin concentrations were directly correlated with hsCRP ($r = 0.8$; $p < 0.001$), ferritin ($r = 0.5$; $p < 0.001$) and EPO dose ($r = 0.4$; $p < 0.05$).
Our data suggest that a dysregulation of hepcidin expression during inflammation may induce an altered iron metabolism and increase EPO requirement in uremic pts, particularly in those treated with HD. Thus, hepcidin may represent a key mediator of anemia of inflammation and an indicator of functional iron deficiency in dialyzed pts.

Erythropoeitin

W-PO40144

EFFECTS OF ERYTHROPOIETIN ON RETINAL CAPILLARY FLOW IN HUMANS

ROLAND E. SCHMIEDER[1], TIM SCHÄUFELE[1], JOANA HARAZNY[2], SEBASTIAN OEHMER[1], CHRISTIAN DELLES[1], GEORG MICHELSON[2]
Department of Medicine / Nephrology, University Erlangen-Nuremberg, Erlangen, Germany[1], Department of Medicine / Ophthalmology, University of Erlangen-Nuremberg, Erlangen, Germany[2]

Background: Erythropoietin (EPO) affects the vascular tone by various pathogenetic mechanisms leading to elevated blood pressure in some subjects. We examined the effect of EPO on retinal capillary flow (RCF) taken as a mirror for cerebral capillary perfusion.
Methods: In an open clinical trial with blind endpoint evaluation 33 subjects (age, 29 ± 5 years) with normal ($n = 17$; 24 hour blood pressure $121 \pm 5/72 \pm 5$ mm Hg) or mildly elevated 24 hour blood pressure ($n = 16$; $136 \pm 7/76 \pm 8$ mm Hg) were enrolled. Scanning Laser Doppler Flowmetry was used to measure RCF before and after intravenous (i.v.) administration of EPO (50 U/kg), and on the next day before and after infusions of *N*G-monomethyl-L-arginine (L-NMMA; 3 mg/kg) and L-arginine (100 mg/kg). After two weeks the protocol was repeated with the same dose of EPO being administered subcutanously (s.c.).
Results: After administration of i.v. EPO, RCF decreased from 344 ± 70 to 322 ± 49 arbitrary units (AU) ($p < 0.05$) in hypertensive but not in normotensive subjects (335 ± 81 vs. 336 ± 71 AU, p = n.s.). This effect on RCF in hypertensive subjects was not found with EPO being administered s.c. (RCF; 339 ± 37 *vs* 342 ± 50 AU, p = n.s.). On the second day after s.c., but not after i.v. administration of EPO, systolic ambulatory blood pressure correlated with the response of RCF to L-NMMA ($r = -0.42$, $p = 0.02$) and to L-arginine ($r = 0.36$, $p = 0.04$).
Conclusions: I.v. EPO caused an immediate reduction in RCF and thereby potentially in cerebral capillary flow in hypertensive subjects. No such effect was observed after s.c. administration of EPO. Thus, EPO should preferentially be administered s.c. in patients with arterial hypertension.

W-PO40145

THE PHARMACOKINETICS OF KRN321 (DARBEPOETIN ALFA) FOLLOWING INTRAVENOUS ADMINISTRATION IN PATIENTS WITH CHRONIC RENAL FAILURE (CRF) RECEIVING HEMODIALYSIS IN JAPAN

AKIRA SUGA[1], SATORU NAGASHIMA[2], KEI HORI[3], TOSHIHIKO UEMATSU[2], EIJI UCHIDA[4], TADAO AKIZAWA[5], SHOZO KOSHIKAWA[6]
Tenjin Medical Office, Fukuoka, Japan[1], Shitoro Clinic, Shizuoka, Japan[2], Munakata Medical Association Hospital, Fukuoka, Japan[3], Showa University School of Medicine, Tokyo, Japan[4], Wakayama Medical University, Wakayama, Japan[5], Showa University Fujigaoka Hospital, Kanagawa, Japan[6]

KRN321 (Darbepoetin alfa) is a hyperglycosylated rHuEPO analogue with two extra carbohydrate chains and has 3 times longer half-life than rHuEPO. The objectives of this study were to investigate the pharmacokinetics and the safety profile of KRN321 following single and long-term multiple intravenous administration in patients with CRF receiving hemodialysis in Japan.
Single-Dose Intravenous Administration: KRN321 was administrated intravenously and blood sample were taken with time to investigate pharmacokinetics. 10, 20, 40 and 60 μg of KRN321 were given for 10 patients in each dose level and 90, 120 and 180 μg were given for 10 patients in intra-patient dose escalating manner (70 patients in total). KRN321 serum concentration increased in proportion to the dose level and diminished with time. AUC was in proportion to the dose level, t1/2, CL, V0, Vss and MRT showed no significant difference by the dose level.
Long-Term Multiple Intravenous Administration: Administration schedule in fourteen subjects with CRF receiving hemodialysis was changed from rHuEPO 2 or 3 times weekly to KRN321 once weekly. The dose of KRN321 was adjusted to maintain patient's target Hb concentration for 28 weeks. Pharmacokinetics was evaluated at the first administration, at 21st week, and at the end of the administration. The serum KRN321 concentrations were measured before the first administration and every 4 weeks. The serum KRN321 concentration profiles at 21st week and at the end were similar to that of the first administration and there was no marked change of pharmacokinetic parameters. The trough serum KRN321 concentration achieved steady state after the 5th administration. From these results, remarkable accumulation of KRN321 by long-term multiple intravenous administration was not observed.
Conclusion: In patients with CRF receiving hemodialysis in Japan, the pharmacokinetic variation of single intravenous KRN321 administration was nearly linear with 10–180 μg and no marked variation in the pharmacokinetics of long-term multiple intravenous KRN321 administration were observed.

W-PO40146

ANTIOXIDATIVE ACTION OF ERYTHROPOIETIN AS A NEUROPROTECTOR IN UREMIC RAT BRAIN

YUKA AGATAHAMA[1], KAZUHIKO TSURUYA[1], HIDEKI HIRAKATA[1], MITSUO IIDA[1], KAZUNORI MINE[2]
Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University[1], Department of Clinical Pharmacology, Graduate School of Pharmaceutical Sciences, Kyushu University[2]

Recombinant human erythropoietin (rHuEPO) improves cognitive function, as assessed by various psychological tests or brain evoked potentials, proportionately with the increase in hemoglobin in anemic patients with chronic renal failure (CRF). Recently, it was demonstrated that erythropoietin mRNA expressed in the hypoxic brain and the exogenously-administered rHuEPO reached and bound its specific receptors on intracerebral capillaries, showing a neuroprotective action. One of its neurotrophic mechanisms may attribute to an antioxidative action. Generation of reactive oxygen species (ROS) is a candidate for the causative factor for uremic encephalopathy, and NADPH oxidase (NOX) is a major origin of ROS in the various tissues including brain in uremia. Thus, we investigate whether rHuEPO acts against ROS in the brain of subtotal nephrectomized rats. Male Sprague-Dawley rats were divided into the following 3 groups; 1) sham-operated rats (Sham), 2) subtotal nephrectomized rats (Nx), and 3) Nx with rHuEPO-treated rats (Nx + rHuEPO). rHuEPO of 500 U/kgBW/day was injected intravenously for 3 consecutive days after 8 weeks of operation, when all rats were sacrificed. Hemoglobin was adjusted around 14 g/dl in all groups by phlebotomy in rHuEPO-treated groups. Accumulation of acrolein, a product of lipid hydroperoxide reaction, and amount of $p67^{phox}$, a cytoplasmic subunit of NOX which translocates to plasma membrane on activation, in the brain in all groups were examined by immunohistochemistry. An activation of NOX was evaluated by the amount of $p67^{phox}$ in the membrane fraction extracted from the whole brain by immunoblot analysis. Immunoreactivity for acrolein and $p67^{phox}$ and the amount of $p67^{phox}$ in the membrane fraction of the brain were increased in Nx compared to Sham. In Nx+rHuEPO, both acrolein accumulation and the amount of $p67^{phox}$ diminished. These results suggest that ROS derived from NOX activation involve in the brain dysfunction in CRF. rHuEPO acts as an antioxidant and may exert a direct neuroprotective action.

W-PO40147

INFLUENCING GENETIC AND ENVIRONMENTAL FACTORS ON ERYTHROPOIETIN RESISTANCE INDEX IN PATIENTS WITH END-STAGE RENAL DISEASE ON HEMODIALYSIS

EUN KYOUNG LEE[1], JAI WON CHANG[2], WON SEOK YANG[2], SOON BAE KIM[2], SANG KOO LEE[2], SU-KIL PARK[2], JUNG SIK PARK[2]
Department of Internal Medicine, Dankook University College of Medicine, Korea[1]; Department of Internal Medicine, Asan Medical Center, University of Ulsan College of Medicine, Republic of Korea[2]

Correction of renal anemia with recombinant human erythropoietin (rhEPO) diminishes cardiovascular morbidity. However, there is a wide individual variation in rhEPO dose to maintain target hemoglobin concentration in current guideline. Angiotensin II (AT II) facilitates EPO production and its circulating level is under genetic control, polymorphism of angiotensin converting enzyme (ACE) gene. Erythropoietin resistance index (ERI, weekly rhEPO dose/hematocrit/body weight) is a collective responsiveness of rhEPO under varied conditions. To investigate genetic and environmental factors affecting ERI in patients with end-stage renal disease (ESRD) on hemodialysis (HD), we obtained serum iron, transferrin, ferritin, albumin, high-sensitivity C-reactive protein, hemoglobin, hematocrit, parathyroid hormone, dialysis adequacy (Kt/V), normalized protein catabolic rate, causes of ESRD, body mass index (BMI), age, sex, duration of dialysis, ACE gene polymorphism (II, ID, DD), ERI, and the administration of ACE inhibitor/AT II receptor blocker. Among 199 ESRD patients (M : F = 94 : 105, age 63 ± 13 years, duration of dialysis 63 months (range, 3 ~ 287months), ERI in the group with DD (n = 25) was significantly lower than that in the group with II (n = 62) or with ID (n = 112)(p = 0.034). There was no significant difference in any other parameters among three groups. In the multivariate linear regression, influencing factors on high ERI were female gender (p = 0.001), lower BMI (p < 0.001), and ACE gene polymorphism of non-DD (p = 0.026). In summary, although ACE gene polymorphism and sex were immutable variables that affect rhEPO requirement dose, we could reduce the ERI through the improvement of nutritional status.

W-PO40148

SUBCUTANEOUS INJECTION (SCI) OF EPOETIN BETA (EB) IS LESS PAINFUL THAN DARBEPOETIN ALFA (DA): A RANDOMISED CROSS-OVER TRIAL IN HEALTHY VOLUNTEERS (HV)

FRANÇOIS BERTHOUX[1], GABRIEL CHOUKROUN[2], STEPHANIE ROUANET[3], AYMERIC DE CHASTEIGNER[4], FRANÇOIS MONTESTRUC[4]
Department of Nephrology, Saint-Etienne, France[1], Department of Nephrology, Amiens, France[2], Therapharm, Boulogne-Sur-Seine, France[3], Roche, Paris, France[4]

Background: SCI remains the recommended route of erythropoietin (EPO) administration for treatment of anemia in advanced renal failure. In Europe, SC EPO treatment is now restricted to EB and DA. The aim of this study was to compare pain after SCI of EB *vs* DA which may affect treatment compliance.
Methods: This was a randomised, single-blind, cross-over trial comparing EB vs DA after placebo (P) in HV. Each subject received at weekly interval SCI of first P (0.3 ml; saline 0.9%) followed by second of EB (0.3 ml; 6000 UI) and third of DA (0.3 ml; 30 µg) or the reverse: second DA and third EB. The primary objective was to evaluate pain experienced both immediately and one hour after SCI with a 10 cms ungraduated visual analogic scale: VAS (no pain = 0; maximum pain = 10) and a 6-item verbal rating scale: VRS (no pain to very intense pain). Forty HV (mean age, 29.9 ± 10.5 y; 21 F) were included but only 37 completed the study. Appropriate statistics for paired studies were selected.
Results: Immediate pain after SCI, quantified by VAS, was 2.77 (95% CI; 1.96–3.58) for DA *vs* 1.15 (0.58–1.72) for EB; p < 0.0001 and 1.38 (0.72–2.04) for P. These results were confirmed by VRS classification: no pain for 16.2% of patients with DA *vs* 51.4% with EB and 51.4% with P contrasting with moderate/severe pain for 37.8% with DA *vs* 5.4% with EB (p < 0.0001) and 13.5% with P. Pain evaluated one hour after SC showed no difference among the groups.
Conclusions: Subcutaneous injection of epoetin beta was perceived by healty volunteers as significantly less painful than darbepoetin alfa and with a comparable pain as with placebo.

W-PO40149

RESPONSE ERYTHROPOIETIN IN CKD PATIENTS ON HEMODIALYSIS WITHOUT IRON SUPLEMENT

ABDURRAHIM RASYID LUBIS[1]
Nephroplogy & Hypertension Division, Department of internal medicine[1]

Background: In Chronic Kidney Disease (CKD) patients decrease production of erythropoietin is the mayor course of anemia, representing low hemoglobine/hematocrite with microcitic normochrome appearance. Treatment by administration of recombinant human eryhropoietin (rHu-EPO) seems to the best alternative but adequate iron supplement is needed for adequate response.
Objective: To evaluate hematologic response and iron status response After the erythropoietin therapy without iron supplement in CKD patients on hemodialisys.
Material and Methods: Subject are CKD patients on hemodialysis, divided by two groups. Group 1 were treated with twice weekly 4000 unit dosage rHu-EPO for 4 week IV. Group 2 were not treated. Both groups did not receive iron supplements. Blood sample are withdrawn at O, 2, 4 weeks after recruitment to determain full blood count and iron status.
Results: Group 1 (14) mean age of the patients was 47.79 ± 8.24 yrs, group 2 (10) 48.70 ± 7.60 yrs. Mean increase (Mean ± SD) hemoglobin in group 1, 10.87 ± 0.98 gr%, group 2, 0.34 ± 1.05 gr% (p = 0.011), hematocrite 2.66 ± 2.66% and 0.69 ± 3.57% respectively (p = 0.012), reticulosit, 0.69 ± 3.57% and 0.02 ± 0.08% respectively (p < 0.0001). Saturasi transferin decrease 11/14 (78.57%) in Group 1, 6/10 (60) in group 2, Ferritin serum 12/14 (85.71%) and 4/10 (40%) respectively. Iron status tend to decrease but did not significant.
Mean decrease (Mean ± SD) Saturasi transferin in goup 1, 6.37 ± 14.25%, in group 2, 0.93 ± 10.11%, (NS), Ferritin serum 180.83 ± 208.17 ug/l and 127.30 ± 281.50 ug/l respectively (NS).
Conclusion: We concluded that increase hematologic response of rH__EPO did not followed by decrease iron status.

W-PO40150

BIOPHYSICAL CHARACTERIZATION AND COMPARISON OF EPOETIN ALFA IN EPREX AND EPOGEN: POTENTIAL IMPLICATIONS FOR UNDERSTANDING THE CAUSE(S) OF PRCA

BRUCE A. KERWIN[1], SONGPON DEECHONGKIT[1], KEN AOKI[2], HEATHER BURNETT[1], SUNGAE PARK[1]
Department of Pharmaceutics, Amgen, Inc.[1], Department of Protein Science, Amgen, Inc.[2]

Recently, Sharma et al.[1] suggested that organic molecules leaching from uncoated stoppers in pre-filled syringe containers act as adjuvanting agents causing the PRCA associated with the polysorbate formulation of Eprex, a recombinant erythropoietic protein manufactured by Ortho Biotech. Coincident with the publication by Sharma was a retrospective analysis by Bennett et al.[2] showing that the human serum albumin formulation of Eprex which does not contain leachates has a rate of 6 cases per 100,000 patient years, only one-third that of the polysorbate containing formulation. Additionally, NeoRecormon, that also contains polysorbate but no leachates is associated with a PRCA rate of 1 case per 100,000

patient years. The discrepancy in rates suggests that other factors, such as the structure of the protein, may be responsible for the cases of PRCA.
Epoetin alfa from the Epogen manufacturing process was formulated in a polysorbate buffer and analyzed by sedimentation velocity analytical ultracentrifugation. Higher-molecular-weight species that were not observed immediately after reformulation were present in the solution after 4 weeks at 4°C. Similar high-molecular-weight species were also observed in commercial samples of Eprex. Additionally, the molecular weight of epoetin alfa in Eprex was approximately 10 kDa higher than expected, suggesting an association of up to 8 monomers of polysorbate per epoetin alfa monomer. Far-UV circular dichroism spectra show a difference in the secondary structure of the proteins. Thermal analyses using the same technique showed that Eprex partially aggregated starting at 70°C while the epoetin alfa of Epogen unfolds reversibly.
While these data do not provide a direct link to the incidence of PRCA they suggest that other factors such as the protein structure may be primarily responsible.

References
[1]Sharma B. *et al Eur J Hospital Pharm.* 2004, 5:86–91.
[2]Bennett, C.L. *et al NEJM*, **2004**, *351*, 1403–8.

W-PO40151

CONVERSION FACTOR OF EPOETIN-ALFA/DARBEPOETIN-ALFA ADMINISTRED INTRAVENOUSLY IN HEMODIALYSIS STABLE PATIENTS

ANDREA ICARDI[1], PAOLO SACCO[1], FRANCESCA SALVATORE[1], UMBERTO ROMANO[1]
Nephrology and Dialysis Unit, Dept. Internal Medicine, La Colletta Hospital, Genoa, Italy[1]

Darbepoetin-alfa (DPO) has different properties and pharmacokinetic profile in comparison with recombinant human erythropoietin (rHuEPO). RHuEPO/DPO conversion factor has been detected using a formula equating the protein mass of the two molecules. However, this dose ratio does not consider rHuEPO formulation (alfa; beta) and the route of administration (i.v; s.c).
Our study looks at the assessment of Epoetin (EPO)-alfa/DPO conversion factor during intravenous administration in hemodialysis (HD) stable patients.
20 HD patients (4 females, 16 males), mean age 63.5 ± 13.9 years, were switched from EPO-alfa maintenance treatment (performed for 6 months) to DPO at extended dosing intervals, using the same route of administration (i.v.) and an initial dose based on peptide mass (200 : 1). I.V. iron supplementation was given to substain serum ferritin levels >200 mg/ml and TSAT >20%; doses of EPO-alfa and DPO were adjusted to maintain stable Hb levels. The observation period was 12 months (before and after switching). The primary end-point was to evaluate the EPO-alfa/DPO ratio every month.
Results are summarized in the Table.

Months	–6	–3	T0	+3	+6
Hb (g/dL)	11 ± 1.1	11 ± 1	11 ± 0.9	11.5 ± 0.9	11.1 ± 0.6
EPO-alfa dose (U/wk)	9375 ± 3774	9473 ± 4659	9250 ± 4265		
DPO dose (mcg/wk)			– 46.3 ± 21.3	41 ± 19.7	37.5 ± 17.8
EPO-alfa/ DPO (U/mcg)			200 : 1	228 ± 24 : 1	247 ± 32 : 1*

*$p < 0.001$

We observe a progressive increase of EPO-alfa / DPO conversion factor, which becomes statistically significant at 6th month (+6) in comparison with starting dose ratio (T0). At the end of the study DPO weekly mean dose shows a reduction of 19%. No significant variations are noted in EPO-alfa weekly mean dose.
Our data indicate that in hemodialyzed patients Hb stability is maintained with low DPO doses than EPO-alfa, when both agents are administered intravenously. This result is consistent with the erythropoietic property of DPO. Unlike rHuEPO, DPO efficacy is independent of the route of administration.

Iron Therapy

W-PO40152

MELATONIN CORRECTS RETICULOENDOTHELIAL BLOCKADE AND IRON STATUS IN HEMODIALYZED PATIENTS

WALTER D. LABONIA[1], DEBORA RUBIO[2], CARLOS ARIAS[2]
Servicio de Nefrología Hospital Santojanni Buenos Aires Argentina.[1], Hospital Santojanni[2]

Treatment of anemia in hemodialyzed patients in the setting of inflammation usually displays high levels of serum ferritin (>800 ng/mL) and low transferrin saturation (TSAT) (<20%) despite i.v. iron supplementation, thus evidentiating iron trapping in the reticuloendothelial system. Melatonin has been reported to reduce cytokine production and, in dialysis patients, to prevent oxidative stress resulting from iron and EPO treatment. In this study, we evaluated a group of 10 patients undergoing hemodialysis who displayed elevated serum ferritin (981 +/– 44.6 ng/mL) and TSAT < 20% (15.6 +/– 3.8%) after having received 1.2 g of i.v. iron dextran over a period of 8 weeks. These patients received oral melatonin, 6 mg/day by night for 30 days. After this treatment, all of them markedly increased TSAT values, reaching 35.5 +/– 6.7% ($p < 0.0001$ vs. basal values). In addition, ferritin values decreased to 754.4 +/– 263.7 ng/mL ($p < 0.05$), and serum iron dramatically increased in all of the patients under study (42.4 +/– 9.4 vs. 109.7 +/– 24.3 mcg/dL; $p < 0.0001$). Values for hematocrit (28.6 +/– 2.7 vs. 31.9 +/– 3.57%; $p < 0.05$) and hemoglobin (9.19 +/– 0.97 vs. 10.04 +/– 1.29 g/dL; $p < 0.05$) were also improved. No adverse effects were observed.
This study demonstrates that melatonin may strongly correct the reticuloendothelial blockade seen in dialysis patients under an inflammatory status, thus allowing a better management of iron derrangements and renal anemia.

W-PO40153

MODEST IRON SUPPLEMENT INCREASES SERUM CARBOXYMETHYLLYSINE (CML) AND MALONDIALDEHYDE-LOW DENSITY LIPOPROTEIN (MDA-LDL) IN HEMODIALYSIS PATIENTS

YASUHIRO ANDO[1], ATSUSHI KOTODA[1], KEISUKE KOTODA[2], YASUSHI ASANO[1], EIJI KUSANO[1]
Jichi Medical School[1], Koganei Clinic[2]

Correction of iron-deficiency is essential to minimize the dose of erythropoietin in hemodialysis patients. Current studies, however, do not agree with the presence of distinct threshold level for serum ferritin below which iron-toxicity, e.g., enhancement of oxydative stress, is negligible. Instead, several lines of evidence suggest that iron supplement may enhance iron-toxicity in parallel with the increase in serum ferritin. In the present study, we examined if iron supplement enhances the production of oxidative substances in hemodialysis patients.
Serum CML and MDA-LDL were measured before and after intravenous administration of chondroitin sulfate-iron colloid (Fe 40 mg/A, 1A/week × 10 weeks). CML ($n = 8$) significantly increased soon after the completion of iron administration and returned to the initial level in 3 months (7.7 ± 0.8 / 9.2 ± 0.7 / 7.4 ± 0.7 μg/ml, before / after / 3 months after, respectively) in parallel with a small increase in serum ferritin (71 ± 11 / 161 ± 32 / 70 ± 18 ng/ml). MDA-LDL increased significantly after iron administration, remaining elevated for 3 months after completion of iron administration (88.9 ± 9.8 / 101.7 ± 19.2 / 107.4 ± 26.7 U/L, $n = 5$) in the patients whose serum ferritin level exceeded 200 ng/ml by iron administration (ferritin; 72.5 ± 13.2 / 280.8 ± 23.5 / 116.5 ± 16.9), while it remained unchanged (74.6 ± 8.3 / 74.7 ± 12.0 / 73.9 ± 9.8 U/L, $n = 9$) in the patients with serum ferritin did not exceed 200 ng/ml after iron administration (ferritin; 54.9 ± 8.1 / 131.7 ± 13.9 / 86.4 ± 26.9 ng/ml).
These data suggest that even modest iron supplement to achieve serum ferritin over 100 ng/ml may enhances oxidative stress in hemodialysis patients. Not only correction of iron deficiency but also iron toxicity should be considered to achieve optimum long-term iron supplement avoiding iron-overload in dialysis patients.

W-PO40154

COMPARISON OF FREE IRON IN FERUMOXYTOL WITH OTHER IRON THERAPEUTICS

JEROME LEWIS[1], PAULA M. JACOBS[1], TIMOTHY B. FRIGO[1]
Advanced Magnetics, Inc.[1]

Transferrin oversaturation and catalytic free iron in serum have been proposed to cause immediate hypotensive adverse events and oxidative stress, respectively. The purpose of this study was to compare different intravenous iron drugs both in vitro and in vivo for free iron and percent transferrin saturation (TSAT).
The catalytic free iron content was determined for four IV iron drugs, sodium ferric gluconate (SFG), iron sucrose (IS), iron dextran (ID), and ferumoxytol, a new agent consisting of an iron oxide core surrounded by a semi-synthetic carbohydrate currently in Phase 3 clinical trials (Advanced Magnetics, Inc, Cambridge MA USA). The bleomycin detectable iron assay (BDI) was used to assay IV-iron compounds added to pooled human serum to give 42 μ grams Fe/ml. Results (μgrams Fe/ml): SFG, 0.405; IS, 0.374; ID, 0.169; and ferumoxytol, 0.080. TSAT and BDI assays were performed on serum of CKD patients receiving one of three different intravenous iron drugs. Patients received a single dose of ferumoxytol at 125 mg Fe ($n = 10$) or 250 mg Fe ($n = 10$) by IV injection, IS at 100 mg or SFG at 125 mg. TSAT values at 5 minutes post dosing are similar for all three intravenous iron drugs. The BDI iron is lowest for the 125 mg dose of ferumoxytol, more than 6-fold lower than the equivalent dose of iron gluconate or iron sucrose, and is dose dependant, approximately doubling with twice the dose of ferumoxytol.
Conclusion: Ferumoxytol has by far the lowest BDI iron in vitro. In vivo, all three iron replacement drugs cause similar rises in TSAT, but ferumoxytol causes approximately 6- fold lower BDI iron than the equivalent dose of iron gluconate or iron sucrose, paralleling the in vitro results.

W-PO40155

LONG-TERM ORAL IRON EFFICIENTLY IMPROVES IRON DEFICIENT ANEMIA AND HYPERPHOSPHATEMIA IN HEMODIALYSIS (HD) PATIENTS

ATSUSHI KOTODA[1], YASUHIRO ANDO[1], EIJI KUSANO[1], KEISUKE KOTODA[2]
Div Nephrol, Dept Intern Med, Jichi Medical School, Tochigi, JAPAN[1], Koganei Clinic[2]

The importance of iron supplement to minimize the dose of recombinant human erythropoietin (rHuEPO) is widely recognized. European and American guidelines recommend intravenous iron supplement, arguing against the efficacy of oral administration up to 200 mg/day. On the other hand, oral iron has been reported to be effective comparably to intravenous administration in chronic uremic patients in conservative period. In Japanese HD patients, evidence against the efficacy of oral iron supplement has been lacking. In the present study, we evaluated the effect of long-term oral iron supplement in chronic HD patients.
Sodium ferrous citrate 50 mg–100 mg/day was orally administrated in 20 HD patients for two years. Laboratory data (blood cell count, serum ferritin) and the dose of rHuEPO were compared at 2th, 6th, 12th 18th 24th month.
While maintaining hemoglobin (Hb) at 10.5 ~ 11.0 g/dl, serum ferritin slowly increased from 47.5 ± 30 ng/ml to 147 ± 72.4 ng/ml** and the dose of rHuEPO reduced from 3637 ± 1095 u/w to 2625 ± 1447 u/w** at 24th month (**: $p < 0.01$ Vs Baseline). This rHuEPO sparing effect was comparable to that of intravenous iron supplement. Additionally, serum inorganic phosphate level significantly reduced from 5.9 ± 1.6 mg/dl to 5.0 ± 0.8 mg/dl in 12 month.
The present study claims for the efficacy of long-term oral iron supplement in HD patients. Despite the absolute superiority of intravenous adminisitration over oral administration in terms of acute iron supplement, several current studies on iron toxicity suggest that iron overload may promote lipid peroxydation and vascular damage in HD patients. Oral iron is safer and less expensive and less laborious than intravenous administration. Oral iron supplement also improves hyperphsophatemia. It is recommended to apply oral iron as a initial mode of iron supplementation even in HD patients.

W-PO40156

ADJUVANT EFFECT OF REGULAR IRON SUPPLEMENTATION IN EPO TREATED IRON-REPLETED HAEMODIALYSIS PATIENTS

VLADIMIR PREMRU[1], MARKO MALOVRH[1]
Deparment of Nephrology, University Medical Centre Ljubljana, Slovenia[1]

Recommended upper serum ferritin (F) and the dose of intravenous (i.v.) iron (Fe) remain uncertain. We explored the possibility to increase the effectiveness of Epo by regular i.v. Fe substitution to HD patients without inducing iron overload.
Patients and Methods: in 52 patients, divided in two groups (A ($N = 27$)-F ≥ 500 mg/l and B ($N = 24$)-F < 500 mg/l), i.v. Fe was applied at 100 mg dose in first half-year period (P1) and 25 mg in second half-year P2. Epo was adjusted to target Hb 120 g/l.
Results: In P1, Fe dose was lower in group A (185 ± 122 mg/mo vs. 370 ± 203 mg/mo; $p = 0.016$). Epo dose was insignificantly lower in group A (6883 ± 4247 IU/wk vs. 7256 ± 3766 IU/wk, NS). In group A, Hb slightly fell (from 124.0 $\pm$ 11.8 to 120.4 ± 10.4 1 g/l, NS) while in group B it rose (from 120.3 ± 10.5 to 123.6 ± 9.2 g/l, NS). At the end of P1, F almost equaled (A : B = 642 ± 333 vs. 661 ± 281 mg/l, NS). Despite smaller Fe dose in P2 vs P1 (117 ± 76 mg/mo ($p = 0.02$) in A and 145 ± 79 mg/mo in B ($p < 0.001$), F rose to 802 ± 175 mg/l in A and to 732 ± 199 mg/l in B ($p < 0.001$). Epo was lower in P2 (13% in A, 23% in B). Cumulative 1 year Epo dose and Epo resistance index were similar. Hb rose to 128 ($p < 0.05$) and Ht to 0.38 ($p < 0.01$) in both groups. Cumulative 1-year Fe was lower in A (1812 mg vs. 3091 mg), but similar in P2 (703 vs. 872 mg).
Conclusions: at steady Epo doses, in iron well-repleted patients without evidence of functional iron deficit, additional improvement of anaemia could be achieved by regular frequent low-dose i.v. Fe. These do not lead to iron overload but only modestly increase serum ferritin. With this approach, 86% of our patients achieved target Hb of 120 g/l. Recommended upper limits of serum ferritin might be underestimated.

W-PO40157

ASSOCIATION BETWEEN HEMOSTASIS IMPAIRMENT AND FUNCTIONAL IRON DEFICIENCY IN HEMODIALYSIS PATIENTS

IRENA PRELOŽNIK ZUPAN[1], MIŠO ŠABOVIČ[2], BARBARA SALOBIR[2], JADRANKA BUTUROVIĆ PONIKVAR[3], PETER ČERNELČ[4], JANEZ LAVRE[5], BOJAN VUJKOVAC[5]
Department of Hematology, University Medical Centre Ljubljana, Slovenia[1], Dept. of Vascular Diseases, University Medical Centre, Ljubljana, Slovenia[2], Dept. of Nephrology and Dialysis, University Medical Centre, Ljubljana, Slovenia[3], Dept of Nephrology and Dialysis, Slovenj Gradec General Hospital, Slovenia[5]

Hemostasis impairment and iron deficiency are relatively frequent in hemodialysis patients. Both conditions may contribute to anemia. The aim of our study was to explore possible associations between hemostasis impairment and iron deficiency by employing recently introduced methods for measurement of both conditions.
Sixty-three hemodialysis patients were studied, with 30 age-matched, healthy controls. Hemostasis impairment was detected by in vitro closure time tests (collagen/epinephrine cartridge: CEPI; collagen/ADP cartridge: CADP), whereas (functional) iron deficiency was measured by reticulocyte hemoglobin content (CHr) and the percentage of hypochromic red cells (HRC).
Patient group ($N = 14$) with functional iron deficiency (CHr < 29) had a significantly delayed in vitro closure times in comparison to the patients ($N = 49$) without functional iron deficiency. Furthermore, both types of closure time (CEPI and CADP) correlated significantly with CHr ($p = 0.002$, and $p = 0.001$). Such an association was not observed between in vitro closure time and HRC.
We found a significant correlation between hemostasis impairment (measured by in vitro closure time) and iron deficiency (measured by CHr) in hemodialysis patients. This correlation has not previously been reported. It is apparent that in hemodialysis patients the hemostasis impairment affects (functional) iron deficiency, probably by facilitating blood loss and consequent iron deficiency. Thus, it seems that a delayed in vitro closure time along with decreased CHr may be characteristic of hemodialysis patients who suffer (occult) blood loss. The clinical value of this finding should be tested in larger studies.

W-PO40158

IRON REPOSITION IN PATIENTS WITH CHRONIC KIDNEY DISEASE (CKD) IN THE PRE-DIALYSIS PHASE

NATALIA FERNANDES[1], RODRIGO REIS ABRITA[1], WANDER BARROS DO CARMO[1], LUIZ CARLOS F. DE ANDRADE[1], MARCUS GOMES BASTOS[1]
NIEPEN-UFJF and Fundação IMEPEN[1]

Anemia is a frequent complication of CKD and has numerous physiologic effects. In the treatment of anemia it is important to guarantee adequate iron stores for hemoglobin synthesis. The aim of this study is to report on the impact of iron reposition on hemoglobin levels in patients with CKD stages 3–5. Forty three anemic patients (hemoglobin < 11.0 g/dL) with CKD stages 3 (16.6%), 4 (58.3%), and 5 (25%), not re-Hu-Erithropoetin were treated with IV iron (Noripurum, Altana Pharma), a ferric sucrose product. Iron deficiency was diagnosed when transferrin saturation was <20% and/or ferritin <100 mg/L. The IV Iron (100 mg/ ampoules) was given in 150 mL saline over 90 minutes. Glomerular filtration rate (GFR) was estimated from serum creatinine using the MDRD formula and patients were staged according the K/DOQI of the NKF. The mean age of the patients was 58.55 ± 17.23 years and 55.8% were female. The main causes of CKD were hypertension (33.3%), diabetes mellitus (19%) and glomerulonephritis (19%). The mean values of the lab results before and after the IV iron administration were: creatinine (2.74 ± 1.28 and 3.01 ± 1.84, p > 0.05), GFR (22.89 ± 9.26 and 25.04 ± 29, p > 0.05), serum iron (58.3 ± 28 and 90.9, p > 0.05), transferrin saturation (24.2 ± 9.8 and 28 ± 8, p > 0.49), ferritin (103.4 ± 92 and 158 ± 126, p < 0.04) and hemoglobin (9.79 ± 1.24 and 10.63 ± 1.44, p < 0.000). The mean number of ampoules was 3.09 (about 300 mg of iron). The IV iron was very well tolerated and no serious side effect was observed. Administration of IV Noripurum, a dextran-free iron compound, was safe and shown a beneficial impact in the hemoglobin level, and its use optimizes the treatment of anemia in patients with CKD.

W-PO40159

ROLE OF SOLUBLE TRANSFERRIN RECEPTORS (STFR) IN UNMASKING IRON DEFICIENT STATE IN PATIENTS WITH CHRONIC RENAL DISEASE (CRD)

PREETI GUPTA[1], SANJAY MEHROTRA[1], SAMEER ATHER[1]
King George's Medical College[1]

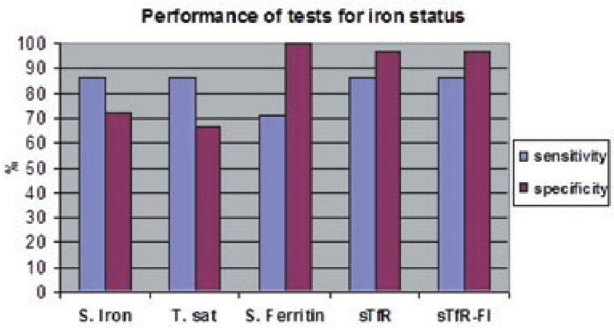
Objective: In patients with CRD classical tests for assessing iron status have yielded mixed.

Results: Bone marrow examination for iron stores remains the gold standard but is an invasive procedure. We have compared sTfR assay with other classical tests of iron profile in reference to bone marrow (BM) iron examination in assessing iron deficient stores in patients with CRD.

Material and methods: Patients with CRD stage 3–5 with anemia were included in this study. Those already on iron replacement or erythropoietin therapy and patients refusing BM examination were excluded. Patients were classified into having either iron sufficient stores (BM iron 3+ or 4+) or iron deficient stores (BM iron 0, 1+ or 2+). Complete blood counts and serum levels of ferritin, Iron, Total Iron Binding Capacity (TIBC) and sTfR were measured in all patients. Values of transferrin saturation and soluble transferrin receptor ferritin index (sTfR-FI) were calculated from the above indices.

Observations and analysis: Of the 43 patients evaluated 7 (16%) had iron deficient stores. The cut off serum values, as determined by ROC curve, were iron < 52, ferritin < 88.9, transferrin saturation > 18.48, sTfR > 5.1 and sTfR/F index > 2.06. TIBC was found to be neither specific nor sensitive and was excluded from comparison. Results are depicted in the figure. Serum ferritin had the highest specificity of 100% (36/36), albeit a low sensitivity (71%). sTfR and sTfR-FI yielded equal results and both had a very high sensitivity and specificity of 86% (6/7) and 97% (35/36). Serum iron had a sensitivity and specificity of 86% (6/7) and 72% (26/36), respectively and Transferrin saturation had a sensitivity and specificity of 86%(6/7) and 67% (24/36), respectively.

Conclusion: Low serum ferritin is diagnostic of iron deficient stores but had a poor sensitivity in patients of CRD. sTfR assay had the highest sensitivity and a very high specificity for predicting iron deficient stores in CRD patients.



W-PO40160

INTRAVENOUS IRON INCREASES LABILE SERUM IRON BUT DOES NOT IMPAIR FOREARM BLOOD FLOW REACTIVITY IN PERITONEAL DIALYSIS PATIENTS

GEORG SCHALLER[1], BARBARA SCHEIBER-MOJDEHKAR[2], MICHAEL WOLZT[1], HEIDI PUTTINGER[3], FRIEDRICH MITTERMAYER[1], WALTER H. HÖRL[3], MANUELA FÖDINGER[4], GERE SUNDER-PLASSMANN[3], ANDREAS VYCHYTIL[3]
Department of Clinical Pharmacology, Medical University Vienna, Austria[1], Department of Medical Chemistry, Medical University of Vienna, Austria[2], Department of Medicine III, Division of Nephrology, Medical University Vienna, Austria[3], Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Vienna, Austria[4]

Background: There are concerns about adverse vascular effects of intravenous iron by inducing oxidative stress. We therefore examined the effect of a single high dose of intravenous iron sucrose on vascular function and biochemical markers of iron homeostasis.

Patients and methods: In a randomized, placebo-controlled, double-blind, parallel-group study, forearm blood flow (FBF) was assessed by strain-gauge plethysmography in 38 peritoneal dialysis patients before and immediately after a single intravenous infusion of 300 mg iron sucrose given over two hours.

Results: Iron infusion increased total (Δ601 μg/100 mL, CI 507, 696) and non-transferrin-bound iron (Δ237.2 μmol/L, CI 173.6, 300.8) approximately ten-fold, as well as redox-active iron nearly five-fold (Δ0.76 μmol/L, CI 0.54, 0.98). FBF response to acetylcholine before and after iron infusion was 263 ± 32% and 310 ± 33%, corresponding to 304 ± 43% and 373 ± 29% in the placebo group, respectively. Before and after iron or placebo infusion, glyceryl-trinitrate increased resting FBF to 232 ± 22% and 258 ± 21% in the iron group, and to 234 ± 18% and 270 ± 30% in the placebo group. L-N-monomethyl-arginine decreased FBF to 70 ± 4% and 72 ± 3% before and after iron, and to 74 ± 4% and 73 ± 4% before and after placebo infusions, respectively. FBF responses were not significantly different between iron and placebo groups.

Conclusions: Our data suggest that high dose intravenous iron sucrose has no acute detrimental effects on FBF in peritoneal dialysis patients, despite a significant increase in non-transferrin-bound and redox-active iron.

W-PO40161

THE EFFECTS OF INTRAVENOUS IRON TREATMENT ON OXIDATIVE STRESS IN CAPD PATIENTS

CANER CAVDAR[1], FUNDA SAGLAM[1], SEZER UYSAL[2], ZAHIDE CAVDAR[2], TANER CAMSARI[1]
Department of Nephrology, Dokuz Eylul University, School of Medicine, Turkey[1], Dokuz Eylul University-School of Medicine-Biochemistry[2]

I.V iron is an accepted treatment in hemodialysis (HD) and CAPD patients. Even some studies demonstrated enhanced oxidative stress in HD patients receiving I.V iron there is no clinical data for CAPD patients.
The aim of this study was to investigate the effect of iron-sucrose on the oxidative status of CAPD patients.
Twelve CAPD patients receiving maintenance I.V iron-sucrose due to NKF-K/DOQI were recruited in this study. Patients were excluded if they were smokers or demonstrated the presence of active infection, acute coronary syndrome, cerebrovascular event prior six months of the study.
After a 12-h fasting, samples were taken for hemoglobin, iron, ferritin, and high sensitive CRP (hs-CRP), baseline activities of erythrocyte (RBC) superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and plasma

malondialdehyde (MDA). 100 mg iron-sucrose (Venofer-Abdi Ibrahim-Turkey) was infused in 30 minutes. Blood samples were taken on 15th, 30th, 60th minutes and 6th hour from an other vein. Statistical analysis was performed and $p < 0.05$ was accepted as significancy.

We found that the plasma iron and transferrin saturation elevated during the infusion. There was no significant change in RBC SOD, CAT, GSH-Px and MDA activities by I.V iron-sucrose infusion ($p > 0.05$). According to the baseline ferritin levels (100–400 and 400–800 ng/ml) the 60th minute MDA was significantly higher in the latter group (400–800 ng/ml) ($p < 0.05$). But it was not significantly higher than the baseline levels ($p > 0.05$). Also there was no correlation between hs-CRP and oxidant-antioxidant balance.

In conclusion there are no acute deteriorating effects of a single dose of I.V 100 mg iron sucrose in CAPD patients with optimal iron stores and without active inflammation. 100 mg I.V iron sucrose/monthly may be a safe dose in CAPD patients.

Others

W-PO40162

ESTIMATION OF RESIDUAL GFR IN DIALYSIS PATIENTS; CYSTATIN C VERSUS MDRDS

FRANS J. HOEK[1], J.C. KOREVAAR[1], F.W. DEKKER[2], E.W. BOESCHOTEN[3], R.T. KREDIET[1],
Academic Medical Center, Amsterdam, NL[1], LUMC, Leiden, NL[2], Hans Mak Institute, Naarden, NL[3]

Introduction: Residual GFR (rGFR) is an important parameter for adequacy in dialysis patients. It requires analysis of urea and creatinine in 24-h urine and in plasma. Collection of 24-h urine is often difficult. Therefore often rGFR is estimated using the MDRDS formula, even though its accuracy in dialysis patients is unknown.

Aim: The potential usefulness of plasma cystatin C (CysC) for estimation of rGFR in hemodialysis (HD) and peritoneal dialysis (PD) patients was investigated.

Methods: A random sample of patients from the Dutch prospective multi-centre NECOSAD study was investigated six months after starting chronic dialysis treatment. Only patients with rGFR >0 and <= 6 mL/min/1.73 m^2 (measured as mean of urea and creatinine clearance) were included. Two third of the patients was assigned to the modelling group to derive a formula for rGFR estimation from CysC, one third was assigned to the validation group. CysC was measured on a BN ProSpec (Dade Behring).

Results: 322 HD and 143 PD patients were included with mean rGFR of 2.7 ± 1.3 (SD) for HD and 3.0 ± 1.4 for PD patients. The formula to predict rGFR from CysC differed only slightly between HD and PD patients. For all patients together it was:

$$rGFR = -0.70 + 22/CysC.$$

In the validation group mean measured rGFR (mL/min/1.73^2) was 2.86 and mean estimated rGFR 3.05 with the CysC formula and 5.99 with the MDRDS formula. Limits of agreement (Bland & Altman) between estimated and measured rGFR were smaller based on CysC than based on MDRDS.

Conclusions: In dialysis patients CysC-derived rGFR is both a more accurate and precise estimate of residual renal function than MDRDS-derived rGFR.

THEME 5: TRANSPLANTATION

Histocompatibility and Immunogenetics

W-PO50001

CORRELATION OF PRETRANSPLANT PRA LEVELS AND PRESENSITIZATION AGAINST HLA CLASS I AND CLASS II ANTIGENS ON GRAFT AND PATIENT OUTCOME IN KIDNEY TRANSPLANT RECIPIENTS

RUBYLAINE GRACE PINOY[1], ROMINA A. DANGUILAN[1], FEDERICK PARALAG[1], FLORECITA PADUA[1]
National Kidney and Transplant Institute[1]

161 primary kidney transplant recipients were retrospectively analyzed to compare graft and patient outcome among patients with PRA levels of <10%, 10–29%, 30–59%, and 60% or higher and to determine whether the presence of pre-transplant PRA levels of 10% or higher to both class I and class II HLA antigens, or to either class I or class II HLA antigens alone, are associated with poorer graft and patient survival at 1 year. Graft survival was highest among patients with a PRA level of <10% (93%) compared to patients with a PRA level of 10–29%, 30–69%, and 60% and higher with survival rates of 91%, 33%, and 67% respectively. A markedly significant decrease in graft survival in patients with an intermediate degree of sensitization (PRA level of 30% to 59%) was found as compared to patients with a PRA level of <30% ($p = 0.001$). There was a statistically significant increase in the episodes of acute rejection ($p = 0.001$) in the former group as compared to the latter. Moreover, rejection episodes appeared earlier. There was no significant correlation between the PRA level and patient mortality or severity of acute rejection. Graft survival at 1 year was significantly lower in patients with a PRA level of 10% or higher to both or either HLA class as compared to patients with a PRA level of <10% to both or either class alone ($p = 0.018$). Acute rejection episodes appeared earlier and more frequent in the former group compared to the latter. Severity of rejection among groups was not statistically significant ($p = 0.130$). Mortality rates at 1 year did not differ between the sensitized and unsensitized groups. ($p = 1.000$).

Conclusion: Pretransplant PRA levels have a negative impact on renal transplant outcome. Presensitization to both or either class of HLA antigens are associated with poorer graft outcome.

W-PO50002

EPIDEMIOLOGY OF PRIOR SENSITIZATION TO CLASS I HLA ANTIGENS IN HAEMODIALYSIS PATIENTS: PRELIMINARY RESULTS

SEAN HAW CHANG[1], WAN TAI SEET[1], SI YEN TAN[1]
University Malaya Medical Centre[1]

The level of panel reactive antibodies (PRA) is an indicator of the degree of sensitization in patients awaiting transplant. We looked at the epidemiology of high PRA levels in our haemodialysis patients.

Methods: Blood samples from 167 unselected patients in 3 haemodialysis centres were analyzed. PRA assay was initially by a low-resolution enzyme-linked immunosorbent assay (ELISA) against panels of Class I and Class II HLA antigens (LATM10X5, One Lambda Inc, Canoga Park, CA). Samples positive for anti-Class I HLA antibodies were further tested against 40 sets of Class I HLA antigens (LAT140, One Lambda Inc, Canoga Park, CA) to determine the percentage of positivity and anti-HLA antibody specificity.

Results: Low-resolution PRA results were positive in 38 (23%) of 165 patients for whom results are available. Of these, 30 had further PRA testing and PRA level >50% occurred in 10 patients. Eight of these were multiparous females (one of whom had a previous renal transplant). Of the remaining 2 males, one had multiple blood transfusions for hereditary spherocytosis. Neither patient gender nor age affected the likelihood of a positive low-resolution PRA (Chi-square tests, $p = 0.460$ and t-test, $p = 0.898$, respectively). In females, number of pregnancies (>2 or =<2) also did not affect the likelihood of a positive low-resolution PRA (Chi-square test, $p = 0.278$). There were 4 patients with previous renal transplants, 3 of whom had positive low-resolution PRA but only 1 of whom had PRA level >50%.

Conclusions: This study suggests that the percentage of sensitized patients in our population is higher than that reported in registries from developed countries. This may be due to higher blood transfusion requirements as accessibility to erythropoietin is more limited. However, these preliminary findings need to be confirmed with a larger group of wait-listed patients.

W-PO50003

SOLUBLE CD30, ANTI-HLA ALLOANTIBODY, AND IGA-ANTI-FAB AUTOANTIBODY AS EXCELLENT IMMUNOLOGICAL MARKERS FOR PRESELECTION OF IMMUNOSUPPRESSIVE REGIMENS

CANER SÜSAL[1], STEFFEN PELZL[1], BERND DÖHLER[1], GERHARD OPELZ[1]
Department of Transplantation Immunology, University of Heidelberg, Heidelberg, Germany[1]

We showed recently that pre-Tx serum sCD30 is an excellent predictor of kidney graft outcome, that the pre-Tx presence of either anti-HLA-class I or -class II antibodies was of no clinical consequence, but that presensitization against both HLA-class I and -class II was associated with poor outcome. We also reported on a graft-protective-effect of IgA-anti-Fab autoantibodies. In the present study we investigated the combined predictive power of pre-Tx sCD30, anti-HLA-class I and II, and IgA-a-Fab. Using these 4 parameters, we were able to classify the recipients into 4 different risk groups. 113 recipients who were positive for both classes of anti-HLA-antibodies (I+/II+) and who had a high sCD30 but low IgA-a-Fab were categorized as "extremely high risk". These patients had a very poor 3-year graft survival rate of 54 ± 5%, compared to the excellent 89 ± 3% rate in the "lowest risk" group of 137 recipients who were negative in one of the anti-HLA-antibody classes and had a low sCD30 but high IgA-a-Fab ($p < 0.0001$). 3-year graft survival in 3552 recipients with negativity in one of the antibody classes, low sCD30, and low IgA-a-Fab was a good 83 ± 1% (low risk), whereas that in 1388 recipients with negativity in one of the antibody classes, high sCD30, and low IgA-a-Fab (increased risk), and in 201 recipients with positivity in both anti-HLA-antibody classes, low sCD30, and low IgA-a-Fab (increased risk) was a lower 72 ± 1% and 70 ± 3%, respectively ($p < 0.0001$). Multivariate analysis confirmed these findings with a low RR value of 0.52 for the "lowest risk" group and a high RR of 2.6 for the "extremely high risk" group. Our data indicate that information on pre-Tx serum sCD30 and anti-HLA antibodies in combination with IgA-anti-Fab can be used to effectively estimate the risk of graft rejection opening a possibility for the selection of appropriate immunosuppression.

W-PO50004

IMPACT OF HLA MISMATCHES ON EFFICACY OF ANTIBODY INDUCTION

SUPHAMAI BUNNAPRADIST[1], FUKAMI S[1], HONG A[1], LEE B[1], TAKEMOTO SK[2]

Cedars-Sinai Medical Center, Los Angeles[1], Health Benchmarks, Inc[2]

Background: Newer antibody induction reduces risk of graft loss in kidney transplantation. We examined 1) whether this benefit was found regardless of level of HLA antigen mismatch (MM), and 2) the relative benefit of Thymoglobulin (Thymo) and anti-IL-2 receptor agonist (IL2RA) induction.

Methods: Of 20,429 deceased donor transplants reported to UNOS between 1999 and 2001, 51% received induction therapy (12% Thymo, 39% IL2RA), 3,239 had 0 ABDR, 4,210 zero DR and 12,980 had DR MM. Propensity scores to indicate likelihood of receiving induction, and hazard ratios for graft loss were adjusted for typical donor and recipient confounding factors.

Results: Recipients with 0 ABDR (propensity score, PS = 0.89, 95% confidence interval 0.82–0.96, P = 0.004) and 0 DR MM (PS = 0.93, 0.87–1.00, P = 0.042) less likely received induction antibody compared to those with DR MM. Induction decreased the risk of graft loss for patients with DR MM (hazard ratio, HR = 0.92, p = 0.008), but not for those with 0 ABDR (HR = 0.90, 0.72–1.14) or 0 DR MM (HR = 0.99, 0.81–1.21). In patients receiving induction, those with 0 ABDR (PS = 0.80, 0.69–0.92) but not 0 DR (PS = 0.98, 0.87–1.10) less likely received Thymo compared to those with DRMM. Hazard ratios for graft loss were not significantly different when comparing induction agents at the 3 levels of MM.

Conclusions: Induction decreased graft loss rates for DR MM deceased donor kidney transplants.

W-PO50005

IDENTIFICATION OF DIALYSIS PATIENTS WITH HIGH PANEL-REACTIVE T CELLS (PRTC) BEFORE KIDNEY TRANSPLANTATION USING AN ALLOGENEIC CELL BANK (ACB)

HOLGER ANDREE[1], CHRISTIN NASIADKO[1], PETER NICKEL[1], GUIDO A. HEYMANN[2], AXEL PRUSS[3], HANS-DIETER VOLK[4], PETRA REINKE[5]

Department of Nephrology, Charité – Hochschulmedizin Berlin[1], Institute of Transfusion Medicine- Charité Berlin[2], Department of Transfusion Medicine-Charité Berlin[3], Institute for Medical Immunology- Charité Berlin[4], Department of Nephrology Charité – Hochschulmedizin Berlin Germany[5]

Background: SOT recipients with donor-reactive antibodies mostly develop hyperacute rejection and enhanced frequencies of donor-reactive memory T-cells predict severe acute rejection and poorer 1-year graft function. Similarly, multiple humoral sensitized patients (PRA+) also express a poorer short- and long-term graft outcome. Nothing is known, however, about multiple T-cell priming. We wondered whether hemodialysis (HD) patients might express panel-reactive T-cell priming to HLA antigens (PRT+).

Methods: Responder T-cells were prepared from healthy volunteers (n = 10) and HD patients with >60% PRA+ (n = 20) or without PRA (n = 21). A stimulator alloreactive cell bank (ACB) was established from peripheral blood non-T-cells of 17 selected HLA-typed healthy blood donors representing the most commonly existing caucasian HLA types. Enriched responder T-cells were incubated separately to each stimulator of the ACB and IFNg-based Elispot was used as described in detail recently.

Results: Using a cut-off of 1/1,000 IFNg+ T-cells, an increased number of HD patients showed allospecific memory response in both the PRA+ (6/20) and PRA– (5/21) group compared to healthy volunteers (1/10). The single detectable response in a healthy volunteer was directed to one ACB donor only, whereas the responses of the HD patients were directed against an average of 2.5 donors. Including also the intermediately strong responders (<1/1,000), 6/10 healthy donors reacted against single donors of ACB, but none of them against a panel (>20%); whereas 12/20 and 7/21 HD-patients with or without PRA were positive, respectively. Most importantly, 6 and 2 patients of the PRA+ and PRA– group, respectively, showed panel-reactive memory T cells (PRT > 20%) suggesting a broad HLA-T-cell priming.

Conclusions: Pretransplant Elispot analyses to our HLA-typed ACB help to identify PRA-negative T-cell presensitized dialysis patients long before transplantation. We suggest to introduce both PRT and memory T-cell "crossmatch" to identify Tx-patients at enhanced risk.

W-PO50006

HLA DIVERSITY IN KIDNEY DONORS AND RECIPIENTS IN KWAZULU-NATAL

ALAIN GUY ASSOUNGA[1], VIOLET SCHREIBER[2], NOMANDLA MADALA[1], THIN HAN[1]

Department of Nephrology, Inkosi Albert Luthuli Central Hospital, University of KwaZulu-Natal, Durban, South Africa[1], Dept of Nephrology Inkhosi Albert Luthuli Central Hospital South Africa[2]

HLA matching of donors and recipients plays a major role in the success of organ transplantation. Race differences in HLA have been reported elsewhere.

Aim: To analyse the diversity of HLA class I and class II among kidney donors and recipients according to race.

Methods: This is a retrospective study of HLA types of 474 renal patients and kidney donors attending the Renal Unit at Addington Hospital from 1985 to 2002. They were 143 Blacks, 169 Indians, 88 Whites and 74 Coloured (individuals of mixed ancestry). Class I HLA typing was done using serological methods while Class II HLA typing was done using serological or molecular **Methods.**

Results: HLA A locus, 18 distinct haplotypes were recorded in Blacks. In Indians, 17 haplotypes were recorded. In Whites, 16 haplotypes were observed and the most frequent were A2(29%) and A1(17%). For the HLA B locus, 29 haplotypes were recorded in Blacks with the two most frequents B58(13%) and B44(12.5%). In Indians, 28 haplotypes were recorded. For DR locus 29 distinct haplotypes were recorded.

HLA type	Blacks (%)	Whites (%)	Indians (%)	Coloured (%)
A2	16	29	20	22
A24	3.5	7.5	17.5	11
A30	19	2.9	1.1	7 (p 0.0001)
B7	8	17	8.8	12
B35	5	6.8	16.4	8.8
B58	13	2.8	1.7	6 (p 0.001)
DR2	3	6	12.6	6.4 (p 0.001)
DR4	6	12.9	11.7	12.8
DR11	17	6.2	4.4	7.2

Conclusion: Race differences in the profile of HLA types are observed. This may render difficult HLA matching between donors and recipients in organ transplantation.

W-PO50007

THE PREDICTIVE VALUE OF FLOW CYTOMETRY CROSSMATCH TO ONE-YEAR GRAFT LOSS FOLLOWING KIDNEY TRANSPLANTATION

RONGZHU WEN[1], GUANGJU GUAN[1], XUEAI LU[1], PAUL KEOWN[2]

Division of Nephrology, The Second Hospital, Shandong University, China[1], Division of Nephrology, Vancouver Hospital, University of British Columbia, Canada[2]

Background: Flow cytometry crossmatch (FCXM) has been extensively used in kidney transplantation, but there is no agreement regarding its predictive value of graft survival following kidney transplantation.

Method: Patients were selected for live donor or cadaveric renal transplantation on the basis of a cytotoxic T-cell crossmatch. FCXM was performed simultaneously, but the results were not disclosed to the transplant team. Logistic model was built to measure the association between flow cytometry crossmatch and one-year graft survival, model variables included early acute rejection, vascular rejection, delayed graft function, FCXM, panel reactive antibody, HLA-A, B mismatch number, HLA-DR mismatch number, donor resource, prior history of transplantation.
Result: A total of 258 kidney transplant recipients from 1997 to 2000 were enrolled; 200 patients received transplants from living donors, and 58 from cadaver donors. Graft loss occurred in 30 subjects during the follow-up, due to death with functioning graft (9), acute rejection (7), primary non-function (3), chronic rejection (4), venous thrombosis (2), acute tubular necrosis (2), technical failure (1), rejection after stopping drugs (1) and uncertain reason (1). 23 graft losses happened in the first year following kidney transplant. Logistic regression showed that significant predictors of one-year graft loss were delayed graft function (odds ratio: 8.00, $p = 0.0014$), cadaver donor (odds ratio: 9.30, $p = 0.0017$) and vascular rejection (odds ratio: 5.05, $p = 0.0219$). FCXM was not a significant predictor with odds ratio of 1.60 ($p = 0.5346$).
Conclusion: FCXM has no predictive value of one-year graft loss following kidney transplantation to CDCXM negative recipients.

Outcomes and Registries

W-PO50008

CLINICAL SIGNIFICANCE OF VESICOURETERAL REFLUX IN RENAL TRANSPLANT RECIPIENTS

HYUN HEE LEE[1], SEUNG MIN YOO[1], JOON SEUNG LEE[1], WOO GYUNG JUNG[1], JONG HO LEE[1]
Internal Medicine, Gachon Medical School, Korea[1]

Background: We investigated the frequency and clinical impact on vesicoureteral reflux (VUR) in the transplanted kidney.
Methods: In this study 55 CsA – treated patients were enrolled who received kidney transplantation between 1993 and 1997. They survived for at least one year while taking Voiding cystourethrography. With the results, we divided into two classes, with and without VUR, where we found such as graft survival, prevalence of hypertension, frequency of urinary tract infection (UTI), creatinine clearance (Ccr) and proteinuria (mg/day) at 1,5,7 years after transplantation.
Results: VUR was detected in 39(71%) patients. Patients with no VUR, the prevalence of hypertension, graft failure, the number of UTI episodes/patient were 16(100%), 1(6.25%), 2.14 ± 0.40 (mean $\pm$ SEM) and patients with VUR were 33(84.6%), 2(5.12%), 1.37 ± 0.26 respectively. There was no significant difference between the two groups ($p > 0.2$). Patients with no VUR, Ccr (mean $\pm$ SEM) at 1, 5, 7 years after transplantation were 69.9 ± 9.1(ml/min), 59.4 ± 3.6, 57.3 ± 3.5 and proteinuria were 171 ± 48.2(mg/day), 188 ± 74.5, 249 ± 119.6 respectively. Patients with VUR, Ccr were 72.0 ± 7.2, 55.5 ± 2.6, 54.1 ± 2.8 and proteinuria were 192 ± 33.8, 148 ± 29.0, 207 ± 74.4 respectively. Also, there was no significant difference between the two groups ($p > 0.4$).
Conclusion: Our study showed that the presence of VUR did not harm graft function or survival.

W-PO50009

PROTEINURIA AS A PROGNOSTIC FACTOR FOR GRAFT SURVIVAL. MOST LATIN AMERICAN STUDY GROUP

PABLO U. MASSARI[1]
Hospital Privado de Córdoba[1]

Introduction: Most is an observational study of renal and liver transplanted patients with Neoral based immunosuppression. Patients were enrolled as 'de novo' or maintenance population categories (all retrospective data since transplantation time were collected for the maintenance category). In Latin America 2195 renal transplanted patients were enrolled from 2001 until 2004. Urinary protein excretion has been previously shown to be a powerful predictor of renal outcome. The information recorded in this large Latin American database was analyzed in order to find out if proteinuria >500 mg/day could be considered also of prognostic value in this population.
Material and Methods: this analysis was performed on the maintenance group: 1073 patients (42 ± 12.4 years old, 42% Caucasian, 57% males), with a mean follow up of 42 ± 23 months after transplantation. Proteinuria was informed as > or <500 mg/day (380 patients). Prognostic value of urinary protein and serum creatinine over graft survival were analyzed using the Cox regression model. ANOVA was performed in order to evaluate the relationship between urinary protein and serum creatinine.
Results: Urinary protein >500 mg/24 hours and/or creatinine >1.5 mg/dl, at one year, were predictors of shorter graft survival $p < 0.0001$ and $p < 0.0049$, respectively. The ANOVA analysis for the interaction between creatinine and urinary protein found higher serum creatinine values ($P = 0.023$) among patients with urinary protein >500 mg/day.
Conclusion: in this population of Latin American renal transplant recipients urinary protein >500 mg/day predicted shorter graft survival and was coincident with serum creatinine increase.

W-PO50010

RENAL TRANSPLANTATION IN JORDAN

RIYAD A. SAID[1]
Renal Transplantation in Jordan, Jordan Hospital Amman[1]

Renal transplantation in Jordan is unique for two reasons; it was the first successful renal transplant to be performed in the Middle East (July 1972), and it was a Cadaveric kidney transplant. Currently almost all renal transplants in Jordan are from Living Related Donors, very few are Cadaveric and no commercial kidney transplant.
Till 1984, all renal transplants were done at the Royal Medical Services (RMS), when the private sector transplant program was started. Currently we have 7 active transplant centers in the country and all of them are in the capital Amman.
Since the start of the renal transplant program in Jordan, a total of 2275 kidneys were transplanted, 2214 were living related and 61 were Cadaveric.
Nearly 90% of the living related kidney transplant were from first degree blood relatives, 4% from second degree relatives and between 5–6% were from spouses.
Almost all patients were maintained on triple immunosuppressive medications; Prednisone, Azathioprine, and Cyclosporine during the ~80 s, and later on both Tacrolimus and Mycophenolate were introduced to the regimen. Induction therapy was not used except in few patients with the second kidney transplant.
The first year results were excellent, both for both patient and the allograft survival (approximately 95%). The incidence of acute allograft rejection has been dropping over the years from nearly 25% during the ~90 s to nearly less than 10% in the recent years.
The study also compares the status of renal transplantation in Jordan with that in the other Arab countries and reviews the current status of the laws and regulation of renal transplant and the need for a National Kidney Foundation in Jordan to promote Cadaveric kidney transplant.

W-PO50011

GRAFT AND RECIPIENT OUTCOME OF CADAVERIC RENAL TRANSPLANTATION AT THE NATIONAL KIDNEY AND TRANSPLANT INSTITUTE FROM 1995–2001

HAZEL DAPHNE NINALGA[1], ROMINA A. DANGUILAN, MD[1]
Nationa Kidney and Transplant Institute (Philippines)[1]

Objectives: This study aimed to determine the graft and patient survival among the recipients of cadaveric renal grafts at the National Kidney and Transplant Institute (NKTI) from 1995 to 2001 and to identify donor and recipient factors that affect graft survival.
Methodology: This is a retrospective cohort study which included 71 adult recipients of primary cadaveric renal transplant. Donor and recipient data were gathered from hospital records and were expressed as ratios, percentages and means with standard deviation. Logistic regression analysis was utilized.
Results: Graft survival at 3 months, 6 months, 1 year and 3 years were 81.25%, 75%, 67.19% and 54.69%, respectively. Immediate graft function was 78.87% while delayed graft function was 15.49%. Four patients (5.63%) had primary graft non-function Patient survival at 6 months, 1 year and 3 years were 84.51%, 78.87% and 67.61%, respectively. Sepsis was the most common cause of death (69.57%). The age, sex, cause of death and terminal creatinine of the donor and the cold ischemia time did not affect graft survival. Among the recipient factors studied (age, sex, coexisting disease, primary renal disease, dialysis type and duration, blood transfusion history, induction therapy, initial immunosuppressive regimen, HLA matching), only diabetes mellitus was significantly associated with a lower graft survival rate. Each increase in the number of HLA mismatch

decreased the graft survival rate by 30–42% at 6 months, 1 year and 3 years, although this was not statistically significant.
Conclusion: Graft survival at 3 months, 6 months, 1 year and 3 years were 81.25%, 75%, 67.19% and 54.69%, respectively Patient survival at 6 months, 1 year and 3 years were 84.51%, 78.87% and 67.61%, respectively. Donor factors did not affect graft outcome. Among the recipient factors, diabetes and the number of HLA mismatches were shown to have some influence in the graft survival.

W-PO50012

RISK FACTORS FOR ALL-CAUSE MORTALITY AFTER SUCCESSFUL RENAL TRANSPLANTATION

YVES VANRENTERGHEM[1], M VILLA[2], S FIEUWS[3]. G MONTAGNINO[2], B MAES[1], C PONTICELLI[2]
UZ Gasthuisberg, Leuven[1], Ospedale Maggiore, Milano[2], Biostatistical Centre, school of Public Health, Leuven[3]

Many remediable and non-remediable risk factors for death after successful renal transplantation have been described. Data of prospectively collected clinical events, routine biochemistry and prescribed drug regimens in 2072 adult renal transplant patients who survived with a functional transplant for at least 1 year in Leuven (Belgium) and Milano (Italy) between 01-01-1983 and 01-01-2000 were analysed. Clinical, biochemical and drug-specific data where collected during the post-transplantation follow-up at month 1, at month 6 and at a yearly basis further on. Multiple Cox regression models were used to analyse the relation between all-cause mortality and the large set of time-constant (baseline data) and time-dependent covariates (follow-up data). Due to the large number of covariates, detection of important predictors proceeded in two steps. First, a parsimonious model for the time-constant covariates has been constructed using a backward-selection strategy. In a second stage, a backward-selection strategy has been performed on the selected set of time-constant predictors extended with the set of time-varying predictors. In the table, the risk factors for death after the first year of renal transplantation are shown. Note that the effect on the hazard for haematocrit and for serum albumin is quadratic instead of simply linear.

Variable	Hazard Ratio	95% CI	P Value
Age (per 1-year increment)	1.072	(1.058, 1.086)	<0.0001
Duration of dialysis (per 1 month increment)	1.006	(1.003, 1.009)	<0.0001
Cardiovascular event after transplantation	1.798	(1.376, 2.349)	<0.0001
Posttransplantation diabetes mellitus	1.771	(1.297, 2.417)	0.0003
Haematocrit			0.0012
Follow-up value	0.737	(0.627, 0.868)	
(Follow-up value)^2	1.004	(1.002, 1.006)	
Pulse pressure (per 1 mmHg increment)	1.011	(1.002, 1.019)	0.014
Corticosteroid therapy (yes)	4.752	(2.795, 8.081)	<0.0001
Number of antihypertensive agents	0.825	(0.743, 0.915)	0.0003
Serum albumin			0.014
Follow-up value	0.177	(0.054, 0.579)	
(Follow-up value)^2	1.254	(1.077, 1.460)	
Follow-up triglyceride level (per 100 mg/dl increment)	1.283	(1.150, 1.432)	<0.0001
Serum creatinine at 1 year (per mg/dl increment)	1.298	(1.048, 1.608)	0.017

All-cause mortality after the first year of renal transplantation relates with pre- and posttransplant cardiovascular risk factors, corticosteroid therapy, posttransplant renal function and posttransplant haematocrit.

W-PO50013

PREDICTIVE FACTORS ON OUTCOME OF RENAL TRANSPLANTATION

CHRISTOPHER TERENCE AGBO[1], JULIA GRAUN[2], THOMAS LORF[3]
Nephrology and Rheumatology, Georg August University Hospital Goettingen Germany[1], Faculty of Medical Science Georg August University Goettingen[2], Dept. of Transplant Surgery Georg August University Hospital Goettingen Germany[3]

There seem to exist some predictive factors that play major role in determining the long term outcome of renal transplantation. The purpose of this study was to compare the various factors that affect the long term kidney transplant survival. The data of all the renal transplantation (144) done in the year 1996 to 2000 in our hospital were analysed and reviewed. The influence of demography (age, sex, body mass index); transplant variables (donor type, panel-reactive antibody titre, time on dialysis, cold ischaemia time, extent of HLA matching); post transplant variables (delayed graft function, immunosuppressive therapy, acute rejection) on patient and graft survival were analysed. Kaplan-Meier estimates and Cox proportional hazards models were used to compare variables.
The overall patient survival rate was 93% at one-year and 81% at 5 years. The overall graft survival rate was 89% at 1-year and 75% at five years. Patient survival rates decreased with a number of HLA mismatch >4 ($p = 0.016$) and delayed graft function ($p = 0.036$). The estimated 5-year graft survival were significantly decreased in a female recipient of a cadaveric donor graft ($p = 0.040$ and $p = 0.027$ respectively). There was almost equal effect by the choices of immunosuppressive regimen (double and triple) with a 1-year patient survival of 94% and 93% respectively. However the use of double therapy increased the patient long term survival significantly ($p = 0.0015$). The 5-year patient survival with double immunosuppressive therapy was 86% while triple immunosuppressive therapy was 69%.
In conclusion, predictive patient and donor characteristics significantly affecting the long term outcome of renal transplantation includes recipient gender, extent of HLA matching, donor type and delayed graft function. The choice of immunosuppressive regimen also play significant role in long term renal transplantation outcome.

W-PO50014

THE CLINICAL OUTCOME OF RENAL TRANSPLANTATION WITH A POOR HLA MATCHED LIVING KIDNEY DONORS

YONG HUN SIN[1], JI HWAN KIM[2], HYAE JU OH[2], YONG KI PARK[2], JOONG KYUNG KIM[2], KIL HUH[3], CHA EUN OH[2]
Department of Internal Medicine, Bong Saeng Hospital, Korea[1]; Bong Saeng Hospital[2], General Surgery, Bong Saeng Hospital[3]

Background: Poor HLA matched donors may become an additional organ source for renal transplantation. This study is conducted to predict the clinical outcomes of renal transplantation in a poor HLA matched group (0 or 1 or 2 HLA matching) by comparing them with those of HLA haploidentical group.
Methods: This study compared a poor HLA matched group (N = 89) with HLA haploidentical group (N = 79) to analyze differences between two groups in graft survival, incidence of acute rejection, cause of graft failure, posttransplant serum creatinine at 1, 2, 3, 5 years. Total 168 cases, appeared in the medical records for more than six months in Bong-Saeng Hospital, from December, 1984 to March, 2004 were traced and identified as relevant cases for this study.
Results: Allograft survival rate at 1, 3, 5, 10 years for poor HLA matched group and HLA haploidentical group were 100%, 98.6%, 95.4%, 72.5% and 100%, 100%, 96.1%, 86.2% (P = not significant). Acute rejection developed in 25.8% of poor HLA matched group versus 18.9% of HLA haploidentical group (P = not significant). The most common causes of graft failure in both groups were chronic rejection.
Conclusion: It should be actively encouraged to consider renal transplantation in a poor HLA matched group as the results of this study support that the clinical outcomes of renal transplantation in a poor HLA matched group are equivalent to those of HLA haploidentical group.

W-PO50015

CLINICAL OUTCOMES IN HLA-IDENTICAL LIVING-RELATED DONOR RENAL TRANSPLANTS

YONG KI PARK[1], CHANG SUE PARK[2], JEE MIN JEON[2], SEONG MIN KIM[2], HYAE JU OH[2], YONG HUN SIN[2], JOONG KYUNG KIM[2], KIL HUH[3], GUN UNG JEON[4]
Internal Medicine, Bong Saeng Hospital, Korea[1], Bong Saeng Hospital[2], General Surgery, Bong Saeng Hospital[3], Dongrae Bong Saeng Hospital[4]

Background: It has been well known that the degree of HLA matching in renal transplantation is important in graft and patient survival. Because HLA-identical living-related donor grafts are free from immunological attacks, they have benefits of one immunosuppressants or early withdrawal of steroids. However, there is acute rejection due to early withdrawal of immunosuppressants and graft loss due to recurrent glomerulonephritis following HLA-identical living-related renal transplantation. The purpose of this study is to determine the graft survival and the impact of recurrent glomerulonephritis on graft survival in HLA-identical living-related donor grafts.

Methods: From December 1984 to March 2004, 44 HLA-identical and 80 HLA-haploidentical living-related renal transplants in Bongsaeng Memorial Hospital were included in this study. We evaluated graft survivals, immunosuppressants and causes of graft failure.
Results: The mean graft survival for HLA-identical transplants is 198 months (16.5 years) and for HLA-haploidentical transplants is 166 months (13.8 years), respectively (p = NS). Acute rejection episodes occurred in 2 of the 44(5%) identical transplants and 17 of the 80(21%) haploidentical transplants, respectively (p = 0.013). 6 grafts were lost in HLA-identical transplants and the causes are 4 recurrent glomerulonephritis (66.7%), 2 chronic rejections (33.4%). 11 grafts were lost in HLA-haploidentical transplants and the causes are 6 chronic rejections (54.5%), 1 acute rejection (9.1%), 1 drug toxicity (9.1%), 3 patient deaths (27.3%). Recurrent glomerulonephritis in HLA-identical transplants are three, but in HLA-haploidentical transplants are none.
Conclusion: Our data revealed that there was no difference in graft survival between the two groups, but lower acute rejection rate in HLA-identical groups. Recurrent glomerulonephritis was the main cause of graft failure in HLA-identical groups and the impact of recurrent disease on graft survival needs to be investigated.

W-PO50016

FACTORS AFFECTING TRANSPLANT OUTCOMES IN PATIENTS WITH END STAGE RENAL DISEASE CAUSED BY LUPUS

BUNNAPRADIST SUPHAMAI[1], PENG A[1], CHUNG P[1], HONG A[1], LEE B[1], FUKAMI S[1], TAKEMOTO S[2], SINGH AK[3]
Cedars-Sinai Medical Center, Los Angeles[1], Health Benchmarks Inc.[2], Brigham and Women Hospital and Harvard Medical School[3]

Objective: We previously demonstrated that patients with Lupus Nephritis (LN) have similar rates of graft survival (GS) and patient survival rates when compared to other causes of end stage renal disease (ESRD). Here we compare the impact of mycophenolate mofetil (MMF) and calcineurin inhibitor (CNI) as well as time on dialysis prior to renal transplantation on transplant outcomes in patients with LN.
Methods: Choice of CNI (cyclosporine (CsA) vs. tacrolimus (tac)), use of MMF (MMF vs. no MMF), and pretransplant dialysis time (greater than 1 year vs. less than 1 year) were examined in renal transplant recipients with ESRD due to LN reported to the Organ Procurement Transplant Network registry between the years 1996 and 2000. GS was compared separately for deceased (DD) and living donor (LD) transplants. Cox proportional hazard models were used to calculate adjusted rates of graft failure (GF), death-censored graft failure (DCGF) and patient death.
Results: A total of 1,959 (1,170 DD and 789 LD) LN transplant recipients were examined. Those receiving MMF had superior 1- and 5-year GS for DD (92.3 vs. 77.5%, 70.4 vs. 59.8%, $p < 0.001$) but not LD (96.4 vs. 90.4%, 77.7 vs. 77.2%, p = ns). The hazard ratio for GF was 0.76 (0.61–0.96) for those receiving MMF compared to no MMF, 0.77 (0.60–0.98) for DCGF and 0.94 for death (p = ns). There was no significant difference in the risk of GF (HR 0.93; 0.72–1.20), DCGF (0.99, 0.75–1.31) or death (0.80, 0.53–1.20) for tac vs. CsA. Pre-transplant dialysis longer than one year did not result in a significant increase in the risk of GF (HR 1.17; 0.89–1.55), DCGF (1.18; 0.87–1.60) or death (1.31; 0.83–2.08).
Conclusions: Recipients with Lupus Nephritis had superior graft outcome when treated with MMF. Time on dialysis prior to transplantation or choice of CNI did not have an impact on graft outcome.

W-PO50017

TACROLIMUS VS CYCLOSPORINE MICROEMULSION: IMMUNOSUPPRESSIVE REGIMEN MAKES A DIFFERENCE IN LIVING RENAL TRANSPLANTATION

ROBERT S. WOODWARD[1], KUTINOVA A[1], BRENNAN DC[2]
University of New Hampshire[1], Washington University[2]

Living donation accounts for over 50% of kidney transplants in the US. Whether immunosuppression regimens with FK or CsA have any cost or outcome advantage in living donor transplant recipients (LDTRs) is unknown.
Methods: We examined complete data sets from the United States Renal Data System (USRDS) on all first, single-organ LDTRs transplanted between 1998–2000 with Medicare as primary payer. We analysed 4 cohorts–patients on 1. FK + MMF + steroids (n = 1,064), 2. CsA + steroids (n = 311), 3. CsA + Aza + steroids (n = 279), or 4. CsA + MMF + steroids (n = 1,854) and the receipt of induction therapy. We used Cox Proportional Hazards regressions to estimate the relative risk of graft survival (GS) and Kaplan-Meier analysis to calculate the average accumulated Medicare payments over 2 years before and after transplantation.
Results: LDTRs on CsA + MMF + steroids had better GS than patients on FK + MMF + steroids (graft loss HR = 0.645, p = 0.037). The relative advantage of CsA + MMF + steroids was strong for patients without induction (HR = 0.493, P = 0.003) but lost its significance for patients with induction (HR = 0.833, p = 0.147). Among patients with induction, recipients of CsA + steroids had superior outcomes compared to recipients of FK + MMF + steroids (graft loss HR = 0.558, P = 0.026). At two years post transplant, the average accumulated Medicare payments were the highest ($70,511) for patients on FK + MMF + steroids, followed by $63,632 for CsA + steroid recipients; $58,729 for patients on CsA + Aza + steroids; and $58,772 for patients on CsA + MMF + steroids. The FK triple regimen was significantly more expensive than any of the CsA regimens (p < 0.001).
Conclusion: Use of CsA + MMF + steroids when compared to FK+ MMF+ steroids in LDTRs was associated with superior GS and lower costs at two years post transplant.

W-PO50018

CONSEQUENCES OF IMMUNOSUPPRESSANT MINIMIZATION FOLLOWING DIAGNOSIS OF GASTROINTESTINAL COMPLICATIONS IN RENAL TRANSPLANT

SUPHAMAI BUNNAPRADIST[1], HARDINGER KL[2], BURROUGHS TE[3], LENTINE KL[3], BRENNAN DC[4], SCHNITZLER MA[3]
Cedars-Sinai Medical Center[1], University of Missouri Kansas City[2], Saint Louis University[3], Washingto university[4]

Minimization of immunosuppression (IMM) is one strategy employed to alleviate post-transplant gastrointestinal complications (GI). However, the consequences of IMM minimization following GI are not fully documented.
Methods: 3,675 adult renal transplant recipients with a Medicare covered MMF prescription at time of GI were drawn from the United States Renal Data System (USRDS) between 1995 and 2001. GI was identified with ICD-9 codes. IMM prescriptions were drawn from Medicare pharmacy bills. The study interval began with GI and ended at graft failure (GF), censoring at 3 years post-transplant, last follow-up, or last IMM record. Impact of various changes in IMM regimens (calcineurin inhibitor (CNI), MMF, and steroids) post-GI were estimated.
Results: Patients treated without CNI at time of GI had increased risk of GF (HR 3.32, p = 0.043). Among CNI-treated patients, treatment with tacrolimus (tac) at GI also had increased risk of GF (HR 4.72, p < 0.0001) compared to cyclosporine (CsA). After GI, CsA dose reduction (HR 2.79, p = 0.013) and CsA withdrawal (HR 3.06, p = 0.011) were associated with increased hazard of GF whereas tac dose changes were not. Following GI, MMF dose reduction >50% (HR 2.36, p = 0.010) and MMF withdrawal (HR 2.72, p = 0.0002) were associated with increased hazard of GF. Returning a patient to their pre-GI MMF dose eliminated the increased risk of GF from dose reduction (p = 0.029). Prednisone dose reduction following GI was associated with a trend toward reduced GF (HR 0.64, p = 0.096).
Conclusions: Outcomes of patients experiencing GI events vary according to IMM regimen at time of GI and its subsequent management. Patients treated without CNI and patients treated with tac (vs. CsA) were associated with a 3 to 5 fold increased hazard of GF. MMF dose reduction and discontinuation following GI diagnosis are also associated with increased risk of GF.

W-PO50019

RENAL ALLOGRAFT OUTCOMES ACCORDING TO INITIAL IMMUNOSUPPRESSIVE REGIMEN: A 5-YEAR FOLLOW-UP OF OPTN DATABASE

BUNNAPRADIST SUPHAMAI[1], PENG A[1], FUKAMI S[1], TAKEMOTO S[2]
Cedars-Sinai Medical Center[1], Health Benchmarks Inc[2]

Objective: Tacrolimus with mycophenolate mofetil (MMF) is now the prevalent regimen for renal transplant patients in the U.S. A previous analysis of Organ Procurement Transplant Network (OPTN) data showed significantly higher 2-year graft survival in living-donor renal transplant patients given cyclosporine-mycophenolate mofetil (CsA-MMF) as initial immunosuppression compared to tacrolimus-MMF (tac-MMF). Here we examine graft outcomes in the same population up to 5 years post-transplant to determine whether this difference continued long-term.

Methods: OPTN data on patients receiving a living-donor renal transplant during 1998–1999 and prescribed either CsA-MMF or tac-MMF as initial maintenance immunosuppression were included, with follow-up through the end of 2003. The propensity to allocate higher-risk patients to tac-MMF was incorporated by calculating a propensity score using covariates that had a significant impact on prescription patterns. Cox proportional hazards analysis including the propensity score was used to adjust for potential confounding factors.
Results: Patients receiving CsA-MMF (n = 4,686) and tac-MMF (n = 2,393) were included. Female recipients, black recipient race, retransplantation and older recipient and donor age were significantly associated with tac-MMF in the propensity analysis. Unadjusted 5-year graft survival was significantly higher with CsA-MMF than tac-MMF (82.1% vs 78.5%, $p < 0.001$). Patient survival was similar in both groups (90% vs 89%, n.s.). After adjustment for potential confounding factors, the hazards ratio (HR) for graft failure at 5 years was significantly higher in patients receiving tac-MMF versus CsA-MMF for both all-cause graft failure (HR 1.23, 95% CI 1.08–1.40, $p = 0.002$) and death-censored graft failure (HR 1.23, 95% CI 1.06–1.43, $p = 0.006$).
Conclusion: Living-donor kidney patients transplanted during 1998–1999 and given tac-MMF as initial immunosuppression had a 23% increase in risk of graft loss compared to those receiving CsA-MMF. A significant difference in graft survival appears to be sustained in more recent patient cohorts.

W-PO50020

NEW PROGNOSTIC MARKERS FOR LATE ORGAN FUNCTION IN RENAL TRANSPLANTATION

CHRISTIAN KOPPELSTAETTER[1], GABRIELE SCHRATZBERGER[1], PAUL PERCO[2], HEINZ REGELE[3], REINHARD KRAMAR[4], RAINER OBERBAUER[3], RAIMUND MAGREITER[5], BERND MAYER[2], GERT MAYER[1]
Clinical Division of Nephrology, Innsbruck Medical University, Innsbruck Austria[1], Emergentec Business Solutions[2], Clinical Department of Pathology, Vienna Medical University, Vienna, Austria[3], Austrian Dialysis and Transplantation Registry, Austria[4], Clinical Department of Transplantation Surgery[5]

Organ shortage has lead to the increasing acceptance of elderly donors in renal transplantation. Although on average late allograft failure is more frequent, organs from elderly donors can maintain excellent function for prolonged periods. Since donor age is not absolutely predictive of outcome, alternative strategies are needed to determine organ quality. Senescence markers like telomeres and cell cycle inhibitors gained importance over the last decade for determining the biological age of an organ. Telomeres, the non-coding regions at the end of chromosomes, are shortened by each cell division as well as by environmental stress factors and represent therefore sensitive markers for biological aging. The cell cycle inhibitors $p16^{INK4a}$ and $p21^{WAF1}$ have been shown to play a prominent role in arresting cells into a senescent state inhibiting renewal and repair of tissue.
44 renal cortical biopsies were obtained prior to implantation. From these tissue samples genomic DNA and RNA were isolated. Relative telomere length and $p16^{INK4a}$ and $p21^{WAF1}$ expression levels were determined by quantitative PCR. Furthermore renal allograft function was assesed as the last available serum creatinine value for the timeframe of 6 to 15 months posttransplant. In an univariate testing model correlating serum creatinine values with donor age, reciepient age, donor gender, acute rejection (+/–), requirement of posttransplant hemodialysis (+/–), relative telomere length and mRNA levels of $p16^{INK4a}$ and $p21^{WAF1}$, donor age achieved the highest predictive value with 33.62% (telomere length 17.25%; $p16^{INK4a}$ gene expression 25.88%). However, performing a stepwise regression model in multivariate analysis including the same parameters revealed that the combination of relative telomere length and $p16^{INK4a}$ and $p21^{WAF1}$ gene expression had the best predictive value with 40.5%. In summary we conclude that these molecular markers exceed the predictive value of donor age significantly and migth be therefore utilised as rapid determinable outcome markers in renal transplantation.

W-PO50021

COMPARISON OF RENAL TRANSPLANTATION IN ASIAN AND CAUCASIAN PATIENTS AT OHSU

JAE SUNG CHUNG[1], JONATHAN C PRATHER[2], MURALI S GOLCONDA[2] DOUGLAS J NORMAN[2]
Nephrology, Siwon Nephrology Clinic, Korea[1]; Transplant Medicine, Oregon Health & Science University, USA[2]

Background: Despite the predominantly Caucasian donor pool, according to the UNOS 2005 annual report Asian recipients had the best five-year kidney graft survival (77.5%), followed by Caucasians (72.4%) and African Americans (59.1%). However, the explanation for this better outcome in Asian renal recipients is not clear. This study was undertaken to investigate the factors that influence better outcome in Asian renal recipients.
Method: The patient group includes 61 Asian renal recipients who received renal transplants at OHSU from Jan. 1985 to Dec. 2000. We selected 61 control Caucasian patients matched based on donor type, number of transplants, recipient age, closeness to date of transplantation, and diabetes as an original renal disease. The patients were followed until April 2002.
Result: Graft survival ($p = 0.680$)and patient survival ($p = 0.943$) were not different between two groups. 6 month and 3 year posttransplant creatinine clearances were comparable. Gender, donor age, gender mismatch, the incidence of PTDM, cardiac events, and status of blood pressure control were also comparable. However, Asians had a greater incidence of donor and recipient racial mismatch (85% vs.11%, $P < 0.0001$), and smaller body weight (60.0 ± 1.6 kg vs.72.9 ± 2.1 kg $p < 0.0001$). Asians tended toward a lower incidence of DGF ($p = 0.230$). HLA mismatch, PRA positivity, incidence of acute rejection, noncompliance, and immunosuppressants were comparable. To see the status of immunologic responsiveness, we investigated steroid withdrawal patients. One of ten Asian and three of twelve Caucasian patients failed steroid withdrawal.
Conclusion: At OHSU, the graft and patient survival rates between two groups were comparable, although Asian recipients had a greater incidence of donor and recipient racial mismatch and lower body weight. Difference in immune responsiveness is an important consideration that remains to be studied.

W-PO50022

PARAMETERS AFFECTING LONG-TERM RENAL ALLOGRAFT FUNCTION AFTER CADAVERIC RENAL TRANSPLANTATION IN CHINESE POPULATION

HOI WONG CHAN[1], KIM MING WONG[1], CHI YUEN CHEUNG[1], YAN LUN LIU[1], YIU HAN CHAN[1], HO SING WONG[1], WAI LEUNG CHAK[1], KOON SHING CHOI[1], KA FOON CHAU[1], CHUN SANG LI[1]
Medicine, Queen Elizabeth Hosptial, Hong Kong[1]

Introduction: To determine the parameters influencing long-term renal allograft function after first cadaveric transplantation, we performed an analytic retrospective study in a single renal transplant center.
Methods: A retrospective analysis was undertaken on records of all patients who underwent transplantation from June 30, 1998 to December 31, 2004. Baseline demographic data were recorded and presented as arithmetic means (±SD). Calculated creatinine clearance (CrCl, ml/min) at 6-month (6 m), 12-month (12 m), 24-month (24 m), 36-month (36 m) and 48-month (48 m) were used as dependent variables. Stepwise general linear model was used to determine the independent association between potential predictor variables and dependent variables. Variables with a correlation ($P < 0.05$) were considered to be statistically significant.
Results: One Hundred cadaveric renal transplants were performed in the study period. Of these, 96 kidney transplants (male : female = 52 : 44) were analyzed after excluding second transplant and technical failure before day-7 post-transplant. Age of patients was 42 +/– 10 years (range, 12–62 years). After a follow-up period of 3.1 +/– 1.9 years, there were four patient deaths (two died of cardiovascular events, one died of carcinoma of stomach, one died of pseudomonas septicemia). There was one graft loss after censored for patient death due to grade 3 rejection. The patient and censored graft survival rates at 3-years post-transplant were 96% and 99%, respectively. Age, sex, cold ischemic time, HLA-mismatch, renal allograft mass normalized to body mass index, types of immunosuppressive regime, had no influence on renal allograft function. The only predictor of long-term graft function was CrCl at 1-month after transplant with a R^2 value at 6 m of 0.62 ($P < 0.001$), 12 m of 0.55 ($P < 0.001$), 24 m of 0.42 ($P < 0.001$), 36 m of 0.36 ($P < 0.001$), and 48 m of 0.28 ($P = 0.023$).
Conclusion: We concluded that baseline CrCl at 1-month after cadaveric renal transplant is independently predictive of long-term renal allograft function.

W-PO50023

EARLY OUTCOMES OF RENAL TRANSPLANTATION IN KWAZULU-NATAL, SOUTH AFRICA

NOMANDLA D. MADALA[1], ALAIN ASSOUNGA[1], MAUNG HAN[2], A HAFFEJEE[2], SARALA NAICKER[3]
Department of Medicine, University of KwaZulu-Natal, South Africa[1], Department of Surgery, University of KwaZulu-Natal, South Africa[2], Renal Unit, University of Witwatersrand, Johannesburg, South Africa[3]

Background: USRDS–UNOS data reported racial disparity in renal transplantation outcomes with best outcomes in Asians. Poorer survival in African-Americans was attributed to greater immune responsiveness, increased frequency of rejection resulting in graft loss, poorer HLA matching, lower socioeconomic status and hypertension.
Methods: We reviewed hospital records of renal allograft recipients from January 1990 to December 2000. We included first time recipients of Indian and African ethnicity. We excluded other racial groups and repeat renal transplants. We collected demographic data, donor and recipient characteristics thought to influence outcome. Donor factors assessed were race and sex. Recipient factors were race, sex, HLA matching, ESRD aetiology, donor kidney source, acute rejection, risk of graft loss and risk of death in the first year post-transplantation. Risk ratio (RR), 95% confidence interval (CI) and chi test were used.
Results: In total, 217 patients met the criteria: 140 (64.5%) were Indian and 77 (35.5%) African. The male : female ratio was 2.4 : 1, mean age was 35.8 years (range 8–57 years). Donor race and sex, recipient sex and HLA matching were similar. There were approximately 60% living donors and almost 40% cadaver donors in both groups. Malignant hypertension was more frequent in Africans and glomerulonephritis more frequent in Indian patients. First year rejection rate was 33.8% in Africans and 32.1% in Indians. Risk of graft loss in the first year post-transplantation was 14.3% (RR 1.0; 95% ~ CI 0.51; 1.58) in both groups and risk of death in the first year was also similar at 7.7% and 7.8% in recipients of African and Indian origin, respectively (RR 0.99; 95% CI ~ 0.38, 2.58).
Conclusion: There were no racial disparities between the two groups in donor and recipient characteristics except malignant hypertension was a significant ESRD cause among South Africans of African origin while glomerulonephritis was more frequent among Indian South Africans. Acute rejection rates, risk of graft loss and death in the first year post-transplantation were similar.

W-PO50024

ICU OUTCOME OF RENAL TRANSPLANT RECIPIENTS AND FACTORS OF PROGNOSIS

KADA KLOUCHE[1], P MASSANET[1], L AMIGUES[1], V GARRIGUE[2], JJ BERAUD[1], G MOURAD[2]
Intensive Care Unit, Lapeyronie University Hospital, France[1]; Renal Transplantation Unit[2]

Though severe transplant-related complications are increasing, their incidence and prognosis have not been extensively assessed. This study was undertaken to evaluate ICU outcome of renal transplant recipients and to identify prognosis factors associated with mortality.
Methods: Records of adult renal transplant recipients admitted to our ICU from 1993 to 2003 were reviewed. Age, sex, underlying medical condition, reason for ICU admission were collected. Apache II, SOFA, and SAPS II scores were estimated 24 h after the admission. Clinical data: mean arterial pressure (MAP), diuresis; biological data: creatininemia, lactatemia, blood urea nitrogen (BUN), albuminemia, and C reactive proteine were collected at the admission. Number of patients requiring mechanical ventilation or/and dialysis and the mortality were assessed. Data were analyzed to identify possible correlation with outcome.
Results: 20 female and 22 males were included. Mean age: 48.7 ± 14.5 yo, Apache II: 30.4 ± 9.2, SOFA: 8.8 ± 4.9, SAPS II: 43.9 ± 16.5. 22 oligoanuric patients, MAP: 98 ± 31 mmHg, BUN: 21.1 ± 12.1 mmol/l, creatininemia: 326 ± 200 micromol/l, lactatemia: 3.6 ± 4.5 mmol/l. Mechanical ventilation: 30 patients (71.4%) and hemodialysis 26 (61.9 %). 19 patients died (45.2%). Survived patients had a significantly lower: Apache II (25.2 ± 7 vs 36.8 ± 7.3), SOFA (5.7 ± 3.5 vs 12.5 ± 3.7), SAPS II (35.5 ± 14.2 vs 54 ± 13.4), and CRP (108.3 ± 77.7 vs 188.5 ± 145.5) than the deceaded. Albuminemia and MAP were significantly higher in survived (28.4 ± 5.3 vs 24.1 ± 5.8 g/l and 115 ± 31 vs 79 ± 18 mmHg). The need of renal replacement therapy or/and of mechanical ventilation significantly worsen the outcome. Among the 23 survived, 9 remained in hemodialysis.
Conclusion: During the study period, the incidence of severe transplant-related complications was at 5.8% (42/725 kidney transplant performed). Indications of ICU admission were mostly related to acute respiratory failure. ICU mortality was at 45%, higher than general ICU mortality (30%). Apache II, SOFA, SAPSII scores; PAM, albuminemia and CRP were all predictive of mortality. Mechanical ventilation and hemodialysis were significantly associated with mortality.

Living Donation

W-PO50025

EFFECT OF ANTIBODY INDUCTION ON GRAFT OUTCOME IN LIVING UNRELATED DONOR KIDNEY TRANSPLANT RECIPIENTS

BUNNAPRADIST SUPHAMAI[1], FUKAMI S[1], PENG A[1], TAKEMOTO[2]
Cedars-Sinai Medical Center, Los Angeles[1], Health Benchmarks Inc[2]

Objective: Living unrelated donor (LURD) kidney transplantation is becoming increasingly common. We investigated the effect of antibody induction on graft outcomes and the relative effect of different antibody types.
Methods: Organ Procurement Transplant Network data for patients receiving a LURD transplant during 1999–2001 were analyzed. Odds ratios (OR) for acute rejection during the first three years post-transplant were calculated using multivariate logistic regression. Hazard ratios (HR) for patient death and graft loss were assessed with Cox proportional hazard techniques. Analyses were adjusted for potential confounding factors, including recipient and donor characteristics, immunosuppression and propensity to receive induction.
Results: 2,162 patients without induction and 2,028 patients with induction were included. Of these, 419 received Thymoglobulin and 1,609 received an interleukin-2 receptor antagonist (IL-2RA). Use of induction was more likely with recipients transplanted in years 2000–01, age <19, cytomegalovirus positive, given mycophenolate mofetil or a retransplant. Unadjusted 3-year rejection rates were 26.2% for no induction, 22.9% with IL-2RA and 17.6% with Thymoglobulin. Adjusted risk of acute rejection was significantly lower with induction vs. no induction (OR 0.85, 95% CI 0.73–0.99, $p = 0.042$). There was a trend to improved death-censored graft survival with induction therapy (HR 0.85, 95% CI 0.67–1.06), but all-cause graft survival was similar.
Of patients receiving induction, Thymoglobulin was more likely administered than IL-2RAs with retransplants, high panel reactive antibody status, or delayed graft function. There was no significant difference in rate of acute rejection between Thymoglobulin and IL-2RA, however patients receiving Thymoglobulin experienced a significantly higher risk of death censored graft loss (HR 1.66, 95% CI 1.11–2.47, $p = 0.013$).

W-PO50026

PRE-EMPTIVE RENAL TRANSPLANTATION: ENSURING BETTER GRAFT AND PATIENT SURVIVAL?

VIJAY KHER[1], R AHLAWAT[1], V SAXENA[1], N H KHAN[1], S JAIN[1]
Indraprastha Apollo Hospital, New Delhi, India[1]

With data suggesting that pre-emptive **renal transplantation** (RTx) has better outcome compared to patients already on dialysis, we have been encouraging pre-emptive RTx at our center during last 5 years. We analyse the results of this group and compare same with those having been on dialysis program before RTx.
72 patients underwent RTx without prior dialysis (preemptive, Group I). 96 transplanted patients had hemodialysis period of less than 3 months (Group II), and 132 were being dialyzed for more than 3 months (Group III) at the time of RTX. Basic characteristics including recipient age, donor age and HLA mismatch were comparable. There were less diabetics in Group I (19% compared to 34% and 40% in Group II and III, respectively). None of the preemptive patients had positive serology for Hep B, Hep C or HIV. Seropositivity for either of these diseases was14% and 17% patients in Group II and III, respectively. Immunosuppression regimen was comparable. Interleukin-2 receptor antibodies were used in approximately one third of patients in each group.
Results:

Parameter	Group I	Group II	Group III
Ac. rejection (Biopsied)	10 (14%)	22 (22%)	30 (24%)
ATN	1	2	1
Patient deaths	0	5	10
Graft survival	100%	91%	90%
1 year patient survival	100%	97%	96%
3 year patient survival	100%	94%	92%
Back on HD (n)	0	2	3

Present day immunosupprsssion provides excellent short term outcome of RTx. 100% three years graft and patient survival in preemptive group highlight that results can be further improved by careful counseling patients for RTx while they are approaching ESRD, and transplanting them as soon as they become symptomatic. Better nutritional status and absence of associated morbidity while on hemodialysis may be the contributing factors.

W-PO50027

THE "TRIALS AND TRIBULATIONS" OF LIVING DONOR TRANSPLANTATION (LDTX) IN SUBSAHARAN AFRICA

ANTHONY MEYERS[1], SANGWENI B, BOTHA JR, MARTINS M, NAICKER S[1]
Depts Surgery & Medicine Johannesburg Hospital and University of Witwatersrand[1]

Lack of cadaver donors (CD) in Black (B) vs Other (Ot) (Caucasian, Mixed, Asian) South Africans is a major problem e.g. 40CDTx in 2004 for a pool of 380 patients awaiting Tx in our centre. We therefore embarked upon a proactive LDTx programme. This paper reports the outcome and costs.
Methods: Demographics, causes and frequency of failed donation (FD) were studied in 2 groups: grp1:1993–02 (n = 356); grp2:2004 (n = 45). Costs estimated for grp2 only. Grp1 was compared to grp2 to assess progress and results in B vs Ot were assessed. Standard workup (WU) tests and routine (Terasaki) plus IgG chromium release (CrR) cross matches (CM) in all. Mean (SD), t tests, X^2 when appropriate.
Results: 132/401 (33%) were B (Total). Grp1:252 = Ot, 104 = B. FD:133 Ot (37%) vs 68 B (66%) ($p < 0.001$). Gp2 17 = Ot, 28 = B. FD:12 Ot (66%) vs 22B (82%). Only 39/166 (21%) LDTx were B (grp1 + 2). FD causes in Table.

Variable	Group 1	Group 2	P-value
Hypertension	25	11	<0.002
Obesity	30	10	<0.04
Renal*	54	2	<0.001
Withdrew	38	4	NS
CM + ve**	24	3	NS
Viral***	16	3	NS
Miscell.	14	1	–

* Renal FD more in Ot vs B. ** Neg Terasaki pos CrR CM. *** 16 HIV; 2 HepB; 1HepC. Hypertension, obesity, withdrawl, viral, significantly more B ($p < 0.05$). (Note: 50–70% ESRF in B = Essential Hypertension. Full Cost:R10.000/WU. Full WU in 47 (13 deferred until 2005), partial (10% cost) in 11. Total = R580.000 i.e 0.75% total hospital budget. Only R110.000 successful LDTx = 19%.

Conclusions: Insufficient CDTx. Not enough LDs for huge pool in B and those who are willing often have a large burden of illness resulting in FD. Hypertension; obesity increasing in B (grp2 vs 1). Cost-ineffectiveness highlighted. Way forward: few short term solutions – B Tx co-ordinator posts required. Long-term requires intensive public education and improved population health and economics.

W-PO50028

PROFILE AND OUTCOME OF LIVING RENAL DONORS: THE UNIVERSITY MALAYA MEDICAL CENTRE (UMMC) EXPERIENCE

KOK PENG NG[1], PROFESSOR SI YEN TAN[1], DR SING HONG LEE[1], SISTER SUAN NEO KOH[1]
University Malaya Medical Centre[1]

Renal transplantation procedure has become a recognized and accepted form of treatment for end stage renal failure (ESRF). Studies had shown that the outcome of living donor kidney transplantation are better than those of cadaveric transplantation, therefore, living kidney donation is advocated in UMMC.
Objective: To study the profile and demographic of living renal donors in UMMC, to examine the peri and post operative complications related to kidney donation and to assess the impact of donor nephrectomy on long term renal function.
Methods: From 1995 to 2004, 82 living donors nephrectomy were performed. All data (details of operation and follow up in outpatient clinic) were obtained retrospectively from patient case notes.
Results: The mean age of living donors was 47 years (range 26–64) and 3 patients were older than 60 years. Mean post operative stay was 5.1 days (range 4–14 days). Complications were seen in 23 donors; 18 were minor and 5 were major. No mortality noted due to nephrectomy**.** The pre- and post-operative values for the variables analysed (based on baseline and 1 year post nephrectomy values) were as follows: creatinine 78.28 ± 13.47 mmol/L (range 53 to 118) and 106.11 ± 23.53 mmol/L (range 65 to 175), $p < 0.001$, urea 3.86 ± 0.90 mmol/L (range 2.2 to 6.7) and 4.91 ± 1.19 mmol/L (range 2 to 9) respectively, $p < 0.001$. In the post operative follow up period of up to 8 years, renal function remained stable in all donors.
Conclusion: There were statistically significant increased of serum creatinine after kidney donations but their kidney function remained stable. The morbidity and mortality rate associated with kidney transplantation in UMMC is low and comparable to other centres.

W-PO50029

RISK OF LIVING KIDNEY DONATION – INDIAN PERSPECTIVE

MANISHA SAHAY[1], GIRISH NARAYEN[2], ANURADHA[2]
Department of Nephrology, Osmania General Hospital, Hyderabad, INDIA[1]*, Osmania General Hospital*[2]

Long term effects of nephrectomy have not been studied in Indian donors. The aim of this pilot study was to review short and long term effects of kidney donation on morbidity, psychosocial outcome and mortality.
Material & Methods: 50 donors who had nephrectomy 3 months to 20 years prior to the study formed the material of this study. Medical history, physical examination, evaluation of renal function pre and post-nephrectomy was done. Quality of life (QOL) was assessed by a standard questionnaire. The effect of age and post nephrectomy duration was studied on renal functions. Data was statistically analyzed.
Results: Males constituted 44% and females 56% of the donors. Parents constituted the majority 39(78%); 10 were siblings (20%) and 1 was a spousal donor. The mean age at donation was 41.26(10.54) years (25–50). Since kidney donation a mean interval of 63 months (3–264 months) had elapsed. There was a mean rise of 9.96 mmHg in SBP & 7.18 mmHg in DBP. Hypertension was noted in 23(46%) and did not show correlation with either the donor age or post nephrectomy duration. 20 donors (40%) developed MAU post nephrectomy and 7(14%) developed overt proteinuria (>300 mg/day), the prevalence of proteinuria correlated well with post nephrectomy interval (71% of proteinuric donors were >10 years post donation). There was a reduction in mean GFR by 28.2(13.57) ml/min after nephrectomy. There was no significant change in serum creatinine after donation, while there was an increase in renal length of 1.14(0.73) cm. None of the donors regretted donation.
Conclusion: While, the long term implications of the fall in GFR, renal hypertrophy, minimal increase in proteinuria and rise in BP need to be further evaluated. this study reaffirms the safety of live kidney donation and underscores the need for initiating a donor registry.

W-PO50030

EFFECT OF DONORS AGE ON LIVING RELATED KIDNEY TRANSPLANT OUTCOME

VISNJA D. LEZAIC[1], RADOMIR T NAUMOVIC[1], JELENA MARINKOVIC[2], LJUBICA D DJUKANOVIC[3], VIDOSAVA NESIC[1]
Dpt. of Nephrology, University Clinical Centre, Serbia and Montenegro[1]*, Institute of Social Medicine, Statistics and Health Research, School of Medicine, Serbia and Montenegro*[2]*, School of Medicine, Serbia and Montenegro*[3]

The present study compared the results of kidney transplantation from living related donors older and younger than 60 years and examined the factors influencing the transplant outcome. Medical records of 273 kidney graft recipients transplanted at our Institute between Jan 1987 and Dec 1999 were analyzed. According to donor age the recipients were divided into group 1–115 patients receiving grafts from older donors (mean age 66.3 years), and group 2–158 patients receiving grafts from younger donors (mean age 49.7 years). The frequency of AR was similar in both groups, but a lower proportion of older graft recipients recovered graft function fully after AR (35% vs. 54%). DGF occurred more frequently in group 1 than in group 2 ($p = 0.001$). The frequency of CRAD was similar in both groups but in the first posttransplant year it was significantly higher in group 1 ($p = 0.002$). While older graft function was mainly influenced by donor age and immunological factors, younger graft function depended on non-immunological factors. Significant better one and five year graft survival for group 2(97% and 71.3%; half-life 120 months) v.s. group 1(88.6% and 55.8%; half-life 84 months) was found ($p = 0.0015$). Risk factors for graft loss were the donor and recipient age difference and AR. One and five year patient survival was 98% and 82% for group 1 and 99% and 93% for group 2 and the differences were significant after the second posttransplant year ($p = 0.002$). Risk factors for patient's death were donor age, the presence of polycystic kidney disease and graft function. In conclusion, kidneys from older donors provide a poorer transplant outcome but, in view of the organ shortage, kidney transplantation from living older donors is an acceptable practice that might be improved by more effective strategies for management of both older donors and recipients.

W-PO50031

RENAL HYPERFILTRATION IN LIVING KIDNEY DONOR

SEBASTIAO FERREIRA-FILHO[1], PABLO SILVA SANTOS[1], EDER VAZ MENDES[1], HENRIQUE VIEIRA LIMA[1]
Internal Medicine, Federal University of Uberlandia, Brazil[1]

In clinical studies, kidney donation for living-related allograft transplantation has been reported as a procedure with minimal long-term risk for the donors, but the effects of reduced renal mass have not been defined. Curiously many clinical published studies compare glomerular filtration rate (GFR) and urinary protein excretion (UPE) obtained in donors before (two kidneys) and after (one kidney) nephrectomy without concerning the values for a one kidney person. We compared the GFR and UPE, before and after nephrectomy in 21 donors. We defined that half of the values for GFR and UPE obtained before nephrectomy was considered the presumable value of each kidney. We studied 12 females and 9 males, 42.3 ± 1.6 yo ($X \pm SEM$). The GFR was estimated by using Cockcroft-Gault formula and the UPE was measured using urine collected in 24 hours before and after kidney donation. The mean time after donation was 7.4 ± 1.2 years and no medications were used by the donors prior this study and they were considered healthy. Our results demonstrated that GFR increased from 50 ± 2.4 to 80 ± 5.1 ml/mim/m^2/per kidney; $p < 0.005$, and the UPE increased from 31 ± 4.4 to 216 ± 41.7 mg/24 hs/per kidney ($p < 0.005$). We conclude that hyperfiltration in the remaining kidney is present after donation and it produces strong increase in the UPE when we compare each kidney. After nephrectomy the total amount of protein excreted is in the normal ranges but is important to note that this excretion is made now by only one kidney and represents an increasing around seven times the initial values.

W-PO50032

CHANGE IN DONOR SERUM CREATININE POST KIDNEY DONATION PREDICTS ALLOGRAFT FUNCTION IN RECIPIENTS

VIRAL S. SHAH[1], JUNAID AHMED[2], LAUREN MALINZAK[2], VANJI KARTHIKEYAN[3], ANITA PATEL[3], M ORTIGOSA-GOGGINS[2], DEAN KIM[2], JUAN ARENAS[2], ATSUSHI YOSHIDA[2], MARWAN ABOULJOUD[2], JOHN JERIUS[2], RAVI PARASURAMAN[2], KK VENKAT[3]
Internal Medicine, Henry Ford Hospital, USA[1]*; Transplantation, Henry Ford Hospital, USA*[2]*; Nephrology, Henry Ford Hospital, USA*[3]

Disparity in nephron mass amongst individuals based on race, age, sex, intrauterine growth restriction and socio-economic status may influence the level of change in serum creatinine post kidney-donation (ΔScr), particularly in African-Americans (AA) and may also influence the recipient allograft outcome.
A single-center retrospective analysis was performed in 61 donor-recipient pairs from 1999–2004 with a mean follow-up of 22 ± 9 months (range: 3–64 months). Donor pool: 34.4% Caucasians, 55.7% AA and 9.9% others. Mean age was 40 ± 10 years. 65.6% were females. No donor had diabetes mellitus; one had hypertension controlled with a single medication without end-organ damage. Family history was significant for diabetes in 36.1% and hypertension in 37.7% patients. Recipient pool: 39.3% Caucasian, 54.1% AA and 6.6% others. Mean age was 48 ± 18 years. 42.6% were females. 86.9% transplants were primary. Immunosupression comprised of cyclosporine ($n = 4$; 6.7%), tacrolimus ($n = 56$; 91.8%), mycophenolate ($n = 59$; 96.7%), steroids ($n = 58$; 95.1%), sirolimus ($n = 1$; 1.7%) and FTY720 ($n = 1$; 1.7%). The incidence of delayed graft function (DGF) was 6.6%. There were 19 episodes of acute rejection (AR) in 14 recipients (23.3%).
The correlation of ΔScr between donors and the mate allograft function are shown in Table. There were no differences in female-to-male and male-to-female donation, HLA-match, induction immunosuppression, DGF and AR between the 2 recipient groups.
Post-donation ΔScr > 50% from baseline in donors correlates with a higher SCr in the recipient. These differences may signify the effect of nephron dosing on subsequent allograft function in the early post-transplant period.

Variable	GROUP I: Donors with ΔScr < 50% (mean 32 ± 9%) Donor-recipient pair n = 34 (55.8%)	GROUP II: Donors with ΔScr > 50% (mean 73 ± 15%) Donor-recipient pair n = 27 (44.2%)	p value
Mean Scr in recipient (mg/dl)	1.9 ± 0.3	2.6 ± 0.2	<0.05
Graft loss (n, %)	2 (5.9%)	4 (14.8%)	ns*
Patient mortality	0	0	ns*

* not significant.

W-PO50033

COMPARISON OF THE INCREASE IN SERUM CREATININE POST KIDNEY DONATION BETWEEN AFRICAN AMERICAN AND CAUCASIAN DONORS

JUNAID AHMED[1], VIRAL S SHAH[3], LAUREN MALINZAK[1], VANJI KARTHIKEYAN[1], ANITA PATEL[3], M ORTIGOSSA-GOGGINS[3], JUAN ARENAS[1], DEAN KIM[1], JOHN JERIUS[1], MARWAN ABOULJOUD[1], ATSUSHI YOSHIDA[3], K K VENKAT[3], RAVI PARASURAMAN[1]
Transplantation, Henry Ford Hospital, USA[1]*, Internal Medicine, Henry Ford Hospital, USA*[2]*, Nephrology, Henry Ford Hospital, USA*[3]

The number of live kidney-donation amongst African Americans (AA) is increasing and the age is shifting towards older donors. These changes necessitate close monitoring of donors for development of hypertension (HTN), proteinuria and chronic kidney disease (CKD), in AA since they are disproportionately afflicted with CKD.
We report our experience with the first 57 donors who donated kidneys between 1999–2004. Mean follow-up period was 22 ± 9 months post-donation (range: 3–64 months). Mean age of donors was 39 ± 8 years, with 20 males (35%) and 37 females (65%). There were 23 Caucasians (40.3%) and 34 AA (59.7%) in the donor pool. One donor (1.7%) had HTN controlled with a single anti-hypertensive medication pre-donation with no end-organ involvement, while there were no diabetic donors. Family history was significant for diabetes in 35% and HTN in 36.5% of donors. Most donors with creatinine of >1.5 mg% during post-donation follow-up had repeat measurements for confirmation.
At a mean follow-up of 22 ± 9 months post-donation, the change in creatinine (ΔScr) between pre-donation baseline and post-donation follow-up was $72 \pm 12\%$ in 26 patients (45.6%), as compared to $31 \pm 7\%$ in 31 patients (54.3%). Table shows donors with change in Scr > 50% from baseline (ΔScr > 50%).
Short-term follow-up of kidney-donors show a significantly higher ΔScr > 50% in AA compared to caucasian donors. This may suggest inadequate compensation of the remnant kidney; however, long-term follow-up with larger number of donors, quantifying GFR by iothalamate clearances, implantation biopsies and monitoring of CKD risk factors may be needed to further validate our findings.

Variable	AA (n = 34)	Caucasian (n = 23)	P value
Predonation Scr (mg/dl); [range, mean]	0.6–1.3; 1 ± 0.1	0.3–1.4; 0.8 ± 0.2	ns*
Postdonation Scr (mg/dl); [range, mean]	0.9–2; 1.5 ± 0.3	0.7–1.9; 1.2 ± 0.2	ns*
ΔScr > 50% (n, %)	18 (75%)	6 (25%)	<0.05

* not significant.

Expanded Donors

W-PO50034

FOLLOW-UP AFTER RENAL TRANSPLANTATION WITH ORGANS FROM NON-HEART-BEATING DONORS (NHBD)

JEREMY CHAPMAN[1], ANDREAS BOCK[2], BERTRAND DUSSOL[2], LUTZ FRITSCHE[2], VOLKER KLIEM[2], YVON LEBRANCHU[2], FEDERICO OPPENHEIMER[2], ERICH POHANKA[2], MAURIZIO SALVADORI[2], GUNNAR TUFVESON[2]

Renal Unit, Westmead Hospital, Westmead, NSW 2145, Australia[1], for the Neoral-MOST study group[2]

Grafts from non-heartbeating donors (NHBD-grafts) have a higher rate of primary non-function or delayed graft function (DGF) compared to grafts from heartbeating cadaveric donors (CAD-grafts), but little is known about their long-term function. The aim of this analysis was to describe characteristics and outcomes of renal transplant recipients whose NHBD-graft remained functional for at least one year post transplantation (Tx).

Methods: MOST is a global observational study in transplant recipients with functioning grafts, receiving cyclosporine microemulsion (CsA-ME) based regimens. The actual data export included 326 evaluable patients who had received a NHBD-graft. As a control group, we choose for each NHBD-patient 1–2 patients with cadaveric heart-beating donors (CAD) from the same centers enrolled into MOST with neighboring transplant dates (N = 340). Calculated Creatinine clearance (Cockcroft) normalized to the BSA was used to estimate GFR. Logistic regression and ANCOVA were used for multifactorial evaluations.

Results: Recipients of NHBD-grafts were of similar age as their CAD-counterparts, their donors tended to be younger (median, 36 vs 38 y), and they received similar immunosuppressive regimen. They experienced a higher incidence of DGF (40% vs 27% in CAD, $p < 0.001$), but their GFR at year 1 as well as their GFR-decline from year 1 onwards were similar to CAD patients (GFR 56 vs. 59 mL/min at 1 year, GFR-decline −1.3 mL/y). Multifactorial analyses confirmed that NHBD did not independently affect GFR at 1 year, while a significant influence was found for donor age and gender, recipient age, DGF, and acute rejection.

Conclusion: In our analysis, no difference regarding demographics or immunosuppression was found between recipients of NHBD-grafts and CAD-grafts. Receiving a NHBD-graft was associated with a higher rate of DGF, but did not independently affect GFR. The majority of NHBD-grafts that survive to one year post transplantation maintain encouraging graft function on a CsA-ME-based regimen.

W-PO50035

ARE THE PAIRED IMPLANT BIOPSIES FROM CADAVERIC DONORS NECESSARY?

SAIT SEN[1], ILKE NALBANTOGLU[1], HUSEYIN TOZ[2], BASAK DOGANAVSARGIL[1], MURAT SOZBILEN[3], MUHITTIN ERTILAV[2], CUNEYT HOSCOSKUN[3]

Ege University, Medical School, Department of Pathology[1], Ege University, Medical School, Department of Nephrology[2], Ege University, Medical School, Department of Surgery[3]

Kidney implant biopsies are used for evaluation of pathologic changes in donor kidneys. But there is no report pointing in necessity of taking paired needle biopsies (PB) from both kidneys of the cadaveric donors. The aim of this study was to investigate the histopathologic changes in cadaveric kidneys and search for the possible differences in biopsies from paired kidneys.

A total of 86 paired cadaveric biopsies from kidneys transplanted to 170 recipients were re-evaluated in respect to chronic histopathologic features such as global glomerular sclerosis (GS), interstitial fibrosis (IF), inflammation (I), tubular atrophy (CT) and arteriosclerosis (determined as intimal fibrosis (CV) and arteriolar hyalinosis (AH)). The severity of changes was scored semi-quantitatively on a scale of 0–3. The sum of the scores was recorded as the chronicity index (CI). The correlation of GS and CI score with age were analyzed as well as the histopathological differences between the paired biopsies.

Mean age of donors and recipients were 30 ± 12 and 37 ± 16 respectively. Non-specific or age related chronic changes were present in 86% of the biopsies whereas 14% showed no histopathologic changes. Severity of histopathological findings and the CI increased significantly with donor age ($p < 0.000$, Pearson correlation). The most disconcordant feature between paired biopsies was CV followed by GS, CI, I, IF, CT and AH (weighted kappa values were 0.20, 0.35, 0.37, 0.37, 0.41, 0.44, and 0.47 respectively).

Implant biopsies are useful in determining baseline pathology. Histopathological findings were more pronounced in older donors. Paired needle biopsies from older donors are valuable as they are supplementing each other. Since kidneys from young donors might have pathological findings; at least one of the kidneys should be biopsied.

Ethics, Legislation, Quality of Life and Economics of Kidney Transplantation

W-PO50036

ATTACHMENT BEHAVIOUR IN CADAVER AND LIVING-RELATED RENAL TRANSPLANTATION

CLAAS LAHMANN[1], SAMANTHA ERMER[1], KARIN TRITT[1], THOMAS LOEW[1], UTE HOFFMANN[2], BERNHARD KRAEMER[2]

Department of Internal Medicine II, Psychosomatic Unit, University Hospital Regensburg, Germany[1], Department of Internal Medicine II, Transplantation Center, University Hospital Regensburg, Germany[2]

Introduction: Severe kidney diseases with kidney transplantation as a therapeutic opportunity obviously have a great influence on the patients family and social environment. Accordingly social networks and, especially, attachment behaviour are of major significance when dealing with kidney transplantation.

Since it has been ascertained that kidney transplantation from living donors result in a substantially better outcome than those from cadaver donors, the question arises whether attachment behaviour in these two groups differs in a clinical relevant degree and therefore might account for the low proportion of living kidney donations.

Methods: In the phase of preparing for transplantation 50 cadaver kidney transplant recipients and 25 from living donors were assessed with a psychometric instrument for attachment behaviour (3 Likert scales: Emotional aloofness, Relationship avoidance, Fear of Loss) in a prospective study covering a 2-year period.

Results: The results confirm the clinical impression that cadaver kidney recipients show difficulties with establishing long-term, stable attachments prior to transplantation.

In accordance, the living donation group showed significantly better values (2.1 points) in the *Relationship avoidance* dimension as opposed to the cadaver donation group (3.2 points). The mean the of living donation group (1.2 points) was significantly better on the *Fear of loss* scale than in the cadaver transplantation group (2.1 points) as well as on the *Emotional aloofness* scale with 1.1 points versus 2.2 points.

Conclusion: As attachment behaviour differs significantly in living donation and cadaver kidney recipients, an early identification of dysfunctional attachment behaviour followed by appropriate psychosomatic intervention could improve interpersonal skills, social function and emotional wellbeing. This in turn potentially could increase the chance of finding living donors. Further research needs to address psychosomatic interventions and their influence on clinical outcome and attendance for living donation.

W-PO50037

QUALITY OF LIFE, COSTS AND RESOURCE MOBILIZATION IN A PUBLIC SECTOR KIDNEY TRANSPLANTATION PROGRAM

JYOTISH CHALIL GOPINATHAN[1], SREELATHA MELEMADATHIL[1], RANJIT NARAYANAN[1], RAMDAS PISHARODY[1]

Calicut Medical College[1]

Background: Data on the costs of Indian kidney transplantation programs and qualitative outcomes are scarce. We studied the socioeconomic status (SES) and quality of life (QOL) of successful kidney recipients, patient and provider costs and pattern of patient resource mobilization in a public sector renal transplantation program.

Methods: Living donor kidney recipients >1 year after surgery with serum creatinine <1.5 mg/dl were grouped into four SES categories using a seven domain scale. Patient costs and resource sources were documented. Provider costs were calculated under capital, overhead and personnel heads. QOL was measured using the short form questionnaire 36 (SF36) and the time-trade off (TTO).

Results: 95% of the 52 patients studied belonged to either lower middle (50%) or low (45%) SES categories. The direct and indirect patient costs (maintenance dialysis, surgery and post transplant medical treatment for one year) showed a median of INR166300. The provider costs for each patient showed a mean of INR 216,903. Community based transplantation facilitation groups were the principal resource sources in 52.5%. SF36 scores performed better in physical health (mean > 75) than in emotional and mental health domains (mean < 70). 40.62% had TTO scores less than 0.5. A significant relationship could be established only between TTO and some SF36 domains (mental health p < 0.001, role playing related to emotional health p < 0.001, social function p < 0.001). No significant relationship was documented between SES and QOL scores (SF 36 p 0.94, TTO p 0.86).

Conclusions: The majority of kidney recipients are socio economically disadvantaged but this did not translate into poor QOL. Suboptimal performances in the mental and emotional health domains indicate psychosocial support needs. Community based resource mobilization can support transplant programs in regions lacking health insurance schemes. This model may be adapted for the viable management of chronic diseases in developing economies.

W-PO50038

IS PHYSICAL TRAINING USEFUL TO IMPROVE THE OUTCOME OF RENAL TRANSPLANT RECIPIENTS (RTRS)?

GIULIO ROMANO[1], BELLUZZO L*[1], TITON I*[1], MONTANARO D°°[1], FERRACCIOLI GF**[1], PERATONER A°[2], SIMONELLA R°[3], MIONI G°°[4], FALLETI E*[5], BORTOLOTTI N*[6], DE IURI E*[7], ANTONUTTO G*[7], DE VITAS[1]

Udine Medical School, Italy[1], UCSC[2], Roma[3], °Pulmonary Rehabilitation Unit[4], A.S.S. "Medio Friuli"[5], and °°Renal Unit[6], Udine City Hospital[7]

Several studies have demonstrated the benefits of rehabilitation in uraemic patients. Our study was carried out to evaluate the physical and psychosocial effects of exercise on RTRs.

Eight RTRs (4 males and 4 females), aged 31–65 years, were evaluated before and after an exercise training session consisting of 30 forty-minute sessions, three times a week, performed with the interval training technique.

The scores regarding both the Hospital Anxiety and Depression Scale (HADS) decreased from 9.1 ± 1.4 to 6.0 ± 1.4 ($p < 0.04$) and from 8.3 ± 1.0 to 5.9 ± 1.6 ($p < 0.008$), respectively. Quality of life mean scores, evaluated through the MOS 36-item short form health survey (SF-36 test), increased from 394 ± 55 to 553 ± 58 ($p < 0.000$). No significant changes were recorded for muscle and fat mass, maximal explosive power of the lower limbs, alkaline and acid phosphatase, PTH, myoglobin, lipoprotein-A, GFR, at rest heart rate and cardiac troponin. On the contrary, the inflammation and bone loss marker IL-6 significantly decreased from 2.8 ± 0.6 to 1.7 ± 0.5 pg/ml ($p < 0.01$). Resting mean arterial pressure fell from 112 ± 4 to 99 ± 3 mmHg ($p < 0.02$). After training, during cardiopulmonary exercise testing, the metabolic threshold rose from 33 ± 4 to $43 \pm 5\%$ ($p < 0.033$), indicating an improvement of the aerobic work capacity, chiefly due to an improved cardiovascular performance. The blood lactate level at peak exercise increased from 5.2 ± 0.9 to 6.2 ± 0.7 mmol/l ($p < 0.012$), consistent with an enhanced anaerobic work capacity. The maximum oxygen uptake increased from 1200 ± 210 to 1359 ± 202 ml/min ($p < 0.05$), while iso-load oxygen uptake decreased from 1110 ± 190 to 1007 ± 187 ml/min ($p < 0.034$). The maximum working capacity increased from 90 ± 14 to 115 ± 15 watts ($p < 0.000$).

Our study suggests that an appropriate dose of physical training is a useful, safe and non-pharmachologic contribution in RTR treatment: exercise, in fact, reduces the risks of cardiovascular disease, improves bone and energetic metabolism and allows for a better quality of life in these patients.

W-PO50039

ECONOMIC IMPACT OF MMF DOSE MODIFICATION FOLLOWING GASTROINTESTINAL COMPLICATIONS IN RENAL TRANSPLANTATION

ROBERT S. WOODWARD[1], SCHNITZLER MA[2], MACHNICKI G[3]

University of New Hampshire[1], Saint Louis University[2], Novartis Pharma AG[3]

Aims: Gastrointestinal complications (GI) are common following renal transplantation. Minimization of MMF coverage is one strategy used to alleviate post-transplant GI. We investigated the economic impact of MMF dose modifications following GI.

Methods: Adult renal transplant recipients with a current Medicare covered MMF prescription at time of GI were drawn from the United States Renal Data System (USRDS) between 1995 and 2001. GI was identified with ICD-9 codes. The study interval began with GI and ended at graft failure (GF), censoring at 3 years post-transplant, last follow-up, or last immunosuppressant record. Using multivariate models, we estimated 3-year graft survival after GI in 3 cohorts of patients, classified according to their MMF prescription pattern in the first 6 months post-GI: (1) No MMF modification (NMM), (2) at least one episode of MMF dose reduction (DR) >30 days, and (3) at least one episode of MMF discontinuation (DC) >30 days. Average Medicare costs were obtained for each cohort. Survival and cost outcomes were integrated in a 3-year Markov model.

Results: 3,675 adult patients were identified with a MMF prescription at GI diagnosis: 59% NMM, 18% DR, and 23% DC. In the first 6 months post-GI, MMF DC was associated with increased risk of graft failure (HR 2.48, $p = 0.031$). In the period beyond 6 months post-GI, the hazard for graft loss was higher for DR (HR = 1.34, $p = 0.019$) and DC (HR = 1.38, $p = 0.038$) groups relative to NMM group. Expected 3-year cumulative costs per patient were $78,367 for NMM group, $87,047 for DR group, $96,584 for the DC group, and $84,119 overall. Reducing the rate of DR and DC by 25% would have lowered expected costs by $5,285,000 in this population.

Conclusions: MMF dose modifications following GI should be managed with great care as they appear to exact long-term economic and graft survival consequences.

W-PO50040

GI-SPECIFIC PATIENT-REPORTED OUTCOME INSTRUMENTS DIFFERENTIATE BETWEEN RENAL TRANSPLANT PATIENTS WITH OR WITHOUT GI COMPLICATIONS

RANDALL FAULL[1], R WALKER[2], R. PRASAD[3], P. AMBUEHL[4], U. BAHNER[5], L. KLEINMAN[6]

Royal Adelaide Hospital[1], The Royal Melbourne Hospital, Melbourne, Australia.[2], St. Michael s Hospital, Toronto, Canada[3], Renal Division, University Hospital Zürich, Switzerland[4], KfH Kuratorium für Dialyse u Nierentransplantation, Würzburg, Germany[5], MEDTAP International, Inc, Seattle, WA[6]

Background: Gastrointestinal (GI) complications are frequently reported by patients post-renal transplant. Mycophenolic acid has been associated with an increased incidence of GI side effects. GI complications may affect patient Quality of Life (QoL). The purpose of this study was to assess the validity of 2 GI-specific and 2 generic patient-reported outcome (PRO) instruments for use in future research.

Methods: The discriminant validity of the Gastrointestinal Symptom Rating Scale (GSRS) and Gastrointestinal Quality of Life Index (GIQLI) was assessed in renal transplant recipients recruited from 5 clinical centers in 4 countries. Patients were receiving a calcineurin inhibitor and mycophenolate mofetil. Patients also completed the Psychological General Well-Being Index (PGWB) and EQ-5D. t-tests and ANOVAs were used to examine differences between patients with and without GI complaints and among differing levels of severity.

Results: Of 96 patients recruited, 41(42%) had none, 35(39%) mild, 12(13%) moderate, and 6(6%) severe GI symptoms. Patients with GI complaints had worse scores on all questionnaires than those without GI complaints. All 5 GSRS subscales and the GIQLI total and 5 subscale scores significantly differentiated between patients with and without GI complications ($p < 0.05$). The PGWB total score, self-control, general health, and vitality subscales and the EQ-5D significantly differentiated between the two patient groups ($p < 0.05$). The generic instruments were unable to discriminate between GI severity levels; conversely, the disease-specific instruments discriminated to some extent between GI severity levels. GSRS abdominal pain subscale discriminated between patients at all levels of severity ($p < 0.05$). GIQLI total score and symptoms subscale discriminated among most severity levels ($p < 0.05$).

Conclusions: The GSRS and GIQLI differentiated between patients with/without GI side-effects and by symptom severity better than generic instruments. They can be used for further research to investigate effects on QoL caused by GI complications due to immunosuppressant therapies.

W-PO50041

DECREASE OF WAITING LIST FOR KIDNEY TRANSPLANTATION IN THE LAST TEN YEARS

IÑIGO MOINA[1], MT. RODRIGO[2], G. GARCÍA-ERAUSKIN[3], R. SARACHO[4], J.J. ARANZABAL[5], J. ARRIETA[1], A. MAGAZ[6]

Basurto Hospital[1], Donostia Hospital[2], Cruces Hospital[3], Galdakao Hospital[4], Transplant Coordination[5], Cohs Consulting In Human Sciences[6]

Objectives: To describe the evolution of waiting list (WL) for renal transplant in dialysis in our community.

Methods: Analysis of the stored data since 1994 by our Renal Patients Registry.
Results: WL has decreased over the last 10 years. The active patients waiting for transplantation were 36% in 1994 and 24% in 2003, with an average age changing from 45 to 50 yr. Temporal contraindication for Tx affected a just 15% in 1994, compared to an actual rate of 35%. Clinical evaluation delay was the reason for temporary contraindication in 15% of patients in 1994, and in 70% in 2003. On the other hand, active diseases as the main reason for Tx transient contraindication decreased from 75% to 25%.
These changes should be analysed jointly with the population's differences at starting RRT. Average age of the incident patients changed from 56 yr to 64 yr over a decade. Primary renal disease distribution has also varied. Diabetes has passed of being 12% of the causes of Chronic Renal Failure (1994) to 15% (2003), with a peak of incidence of 22% (2002). The most remarkable change in primary renal disease among patients starting RRT in our region was glomerulonephritis rate, passing from 21% in the yr 1994–1998, to 15% in the last five years.
The median of the time waiting for Tx for our dialysis patients have decreased from 1.5 yr to less than a year, considering the date of activation in WL and nor the date of starting dialysis.
Conclusions: We can observe a decrease of active patients in wait list in the last ten years. A lower rate of indication of Tx and an increasing delay in activation after starting dialysis are the main parameters that might justify this evolution of our WL.

W-PO50042

NUTRITIONAL STATUS IN KIDNEY TRANSPLANT PATIENTS

LIIDIA KIISK[1], SIIRI/MESIKEPP[2], ELVIIRA/SEPPET[2], MAI/OTS[2]
Anatomy University of Tartu, Estonia[1], Internal/University of Tartu/Estonia[2]

Aim of the study: was to compare nutritional status in kidney transplant (tx) patients (pts) before and one year after the transplantation.
Material and methods: 28 pts were studied: 12 males and 16 females. The following anthropometrical methods were assessed: body weight (kg) and height (cm), Body Mass Index (BMI); body fat mass (%) and total body fat mass in kg by hand- hand bioimpedance Omron BF 300 body mass monitor, seven heights; ten breadth and depth measurements, 13 circumferences, 12 skinfolds, total body composition with dual-energy X ray-absorptiometry (DEXA). Biochemical indices included serum albumin, creatinine, calculated GFR and lipid profile. Dietician systematic reviews carried out in every patient.
Results: showed that the nutritional status improved after one year from the cadaver kidney transplantation compared with the pretransplant period.

Table. Nutritional Status in transplanted patients

	I	II	I	II	I	II	I	II
	Body Fat %	Body Fat %	Body Fat kg	Body Fat kg	BMI kg/m^2	BMI kg/m^2	WHP	WHP
Males n = 12 Age 18–70 (42.7)								
Min	7.8	8.16	5.5	6.43	19.4	22	0.81	0.8
Max	39.4	38.1	52.8	50.8	46.1	45.9	1.01	1.13
Mean	20.73	23.48	16.38	20.09	24.56	27.51	0.90	0.93
Females n = 16 Age 21–71 (46.3)								
Min	11	11	4.4	5.1	14.9	17.2	0.70	0.69
Max	46.3	47.9	57.3	59.8	42.3	44.5	1	0.93
Mean	34.28	35.9	27.09	29.2	27.6	29	0.83	0.81

Conclusion: Nutritional status assessment is important in the complex rehabilitation and management of kidney transplant pts. Studied pts were in good nutritional status after the transplantation, which may be associated with regular monitoring, cooperation of pts with treatment and with good collaboration between specialists. Nnutritional counselling should be continued to avoid overweight.

W-PO50043

TREATMENT OF ERECTILE DYSFUNCTION WITH SILDENAFIL CITRATE IN RENAL TRANSPLANT RECIPIENT: A CROSS OVER PLACEBO CONTROLLED TRIAL

RAJ KUMAR SHARMA[1], NARAYAN PRASAD[1], R KAPOOR[2], AMIT GUPTA[1], S GULATI[1],
Nephrology, SGPGIMS, lucknow, India[1], Urology, SGPGIMS, lucknow, India[2]

Sildenafil is an effective modality of treatment of Erectile dysfunction (ED) in normal population. We conducted a study to assess safety and efficacy of sildenafil in renal transplant patients with ED.
Thirty recipients with erectile dysfunction were enrolled for, cross over, double blind, placebo controlled study. Sildenafil was given in dose of 50 mg 1 hr before sexual activity and completing International index of erectile function (IIEF) score performa. Placebo and sildenafil were used in blinded manner. Outcome was analyzed in terms of improvement in IIEF score and global efficacy question. Mean IIEF score at baseline and after treatment with sildenafil were followings (1) frequency of erection (2.2 ± 0.6 vs 3.7 ± 0.7, $P < 0.001$), (2) frequency of erections hard enough for penetration (1.8 ± 0.7 vs 3.5 ± 0.6, $P < 0.001$), (3) penetration ability (2.2 ± 0.7 vs 3.9 ± 0.6) $P < 0.001$), (4) maintenance frequency (2.1 ± 0.9 vs 3.8 ± 0.6, $p < 0.006$), (5) maintenance ability (1.7 ± 0.84 vs 3.7 ± 0.9, $p < 0.001$), (6) intercourse frequency (1.3 ± 0.6 vs 2.9 ± 1.1, $p < 0.001$), (7) intercourse satisfaction (1.7 ± 0.8 vs 3.6 ± 1.8, $p < 0.001$), (8) intercourse enjoyment 2.1 ± 0.9 vs 3.7 ± 0.7, $p < 0.001$, (9) ejaculation frequency (2.1 ± 1.2 vs 4 ± 0.9, $P < 0.001$), (10) Orgasm frequency (2.1 ± 1.1 vs 3.7 ± 0.7, $p < 0.001$), (11) desired frequency (3.2 ± 1.2 vs 3.5 ± 0.6, $P < 0.32$), (12) desire level (2.8 ± 0.8 vs 3.1 ± 0.8, $P = 0.6$), (13) Overall satisfaction (2.5 ± 0.7 vs 3.7 ± 0.5, $p < 0.001$), (14) Relationship satisfaction (2.7 ± 0.8 vs 3.5 ± 0.6, $p < 0.001$), (15) erectile confidence (2.6 ± 0.8 vs 3.67 ± 0.6, $p < 0.001$). IIEF score was similar pre and post placebo therapy. On global efficacy analysis, 26/30 patients had significant improvement in erectile function after sildenafil therapy. There was no change in graft function (Creatinine 1.48 ± 0.36 vs 1.4 ± 0.39 mg/dl) and CsA level (90 ± 9.9 vs 91 ± 10.5 ng/ml) pre and post sildenafil treatment. In selected 4 patients, AUC of cyclosporine 7454 ± 1483 vs 7460 ± 1480 ng-hour/ml was similar with and without sildenafil. None of patients discontinued drug due to side-effects except one with visual symptoms.
Sildenafil is a safe and effective therapy for ED in renal transplant patients.

Paediatric Transplantation

W-PO50044

URINARY TRACT INFECTION AFTER KIDNEY TRANSPLANTATION IN CHILDREN: INCIDENCE AND OUTCOMES

VIKAS DHARNIDHARKA[1], KEVIN ABBOTT[2], LAWRENCE AGODOA[3]
University of Florida[1], Walter Reed Army Medical Center[2], NIDDK[3]

Background: UTI is the most common infection post kidney transplant. In a previous analysis, we have shown that late (>6 months post transplant), but not early UTI, is associated with earlier graft loss in adults. We hypothesized that children <17 years old would be at higher risk to develop UTI than adults and for early graft loss after both early and late UTI.
Methods: We analyzed the USRDS database from 1995 to 2001 for Medicare claims (composite of inpatient and outpatient) for UTI up to 36 months post transplant. SPSS software and Cox regression models were used to determine association of UTI and age after adjustment for covariates.
Results: In comparison to adults 18–49 years, the risk for earlier graft loss after early UTI was elevated in all children, (RR 3.34, 95%CI = 1.39, 8.03, $p = 0.007$), but not after late UTI (RR 1.72, 95% CI 0.48, 6.19, $p = 0.4$). Risk of post transplant death was not significantly increased after early UTI (RR 1.23, 95%CI = 0.37, 4.08) or late UTI (RR = 2.22, 95% CI = 0.90, 5.44).
In a subset analysis, using 18–49 year adults as the reference group, males in the 2–6 and 6–12 year age groups were at higher risk for hospitalization, RR 2.47 and 1.69, respectively, $p < 0.03$ for both. In females, the two youngest age categories of 0–2 had higher risk for UTI hospitalization (RR 4.29, $p = 0.04$) but not the older age categories.
Conclusions: In contrast with our previous results in adult transplant recipients, children are at greater risk for graft loss after early, but not necessarily late, UTI, while UTI was not an independent predictor of death.

W-PO50045

FLUID BALANCE IN FIRST WEEK AFTER KIDNEY TRANSPLANT CHILDREN

ADISORN LUMPAOPONG[1], EUNICE JOHN[1], JOSE OBERHOLZER[2], GIULIANO TESTA[2], HOWARD SANKARY[2], ENRICO BENEDETTI[2]
Pediatrics, University of Illinois at Chicago, Chicago, IL, USA[1], Transplant Surgery, University of Illinois at Chicago, Chicago, IL, USA[2]

Background: Postoperative fluid management in kidney transplant children is one of the important roles for postoperative care. Underestimated fluid management may cause dehydration, inadequate urine output, and slow improving creatinine clearance. In this study, we retrospectively reviewed postoperative fluid management and fluid balance in kidney transplant children.
Material and Method: Thirteen patients age 10.5 ± 5 years (range 1–17 years) were included in this study. Eleven patients received thymoglobulin (5–7 days) and two patients received basiliximab (day 0 and 4) as induction. All patients underwent fluid management to maintain central venous pressure (CVP) 8–12 cmH_2O. Type of intravenous fluid was adjusted based on serum and urine electrolyte. Fluid balance was evaluated by daily intake, output and insensible water loss.
Result: Urine replacement cc by cc and fluid bolus was given in all patients to maintain CVP and vital sign in the normal range. Positive fluid balance was observed in first 2.2 ± 0.6 days (range 1–3 days) posttransplant. After 2.7 ± 0.9 days (range1–4 days) posttransplant, urine replacement was gradually decreased due to positive cumulative fluid balance and increasing CVP. Most patients had zero cumulative balance and reached to baseline body weight within 5.6 ± 1 days (range 4–8 days). Creatinine and creatinine clearance at first week was 0.83 ± 0.6 mg/dl and 114.5 ± 45 cc/min/1.73 m^2 respectively.
Conclusion: Positive fluid balance was common in early posttransplant period. The mechanisms might associated with increasing vascular permeability from surgery and immunosuppressive drug. Monitoring intake, output and calculation fluid balance was useful for deciding fluid management in kidney transplant children.

W-PO50046

ONE YEAR FOLLOW UP IN RENAL TRANSPLANT CHILDREN WITH TWO DIFFERENT IMMUNOSUPPRESSIVE REGIMENS

RODOLFO DELGADILLO[1], MARA MEDEIROS[1], BENJAMIN ROMERO[1], REBECA GOMEZCHICO[1], ANA MARIA HERNANDEZ[1], GUILLERMO RAMON[1], SAÚL VALVERDE[1], GUSTAVO VARELA-FASCINETTO[1], RICARDO MUÑOZ[1], FELIPE MOTA[1]
Departmento De Nefrologia, Hospital Infantil de Mexico Federico Gomez[1]

Renal transplant is the ideal therapy for end stage renal disease children. New and more potent immunosuppressive agents are now available. Nevertheless, the decrease of rejections can accompany of increase of immunosuppressive therapy complications, as lymphoproliferative disorders.
Methods: A descriptive, retrospective study was performed in children transplanted between January 1993 to Dicember 2003. Two different immunosuppressive methods were used: Group I prednisone (PDN), Azathioprine (AZA) and calcineurin inhibitor (CI), Group II Basiliximab, prednisone, Sirolimus and calcineurin inhibitor. The one year follow up was compared in the two groups.
Results: 223 children were transplanted during the study period. Two children were excluded because they switched to another regimen. 163 received treatment Group I and 60 treatment Group II. General results are shown in table 1.
Conclusions: Patients in Group II had lower incidence of acute and chronic rejection but a higher incidence of PTLD and a higher serum creatinine at one year follow up.

Experimental Transplantation

W-PO50047

THE EFFECT OF CYCLOSPORINE ON CHRONIC ALLOGRAFT CHANGES IN SYNGENIC KIDNEY TRANSPLANTS

JOHANNA SAVIKKO[1], EVA VON WILLEBRAND[1]
Transplantation Laboratory, University of Helsinki, and Helsinki University Central Hospital[1]

Aim: Chronic allograft nephropathy is still the major reason for late allograft loss in clinical kidney transplantation. Cyclosporine (CsA) has dramatically decreased the incidence of acute rejection, and prolonged the early survival of kidney transplants. However, the long-term survival of kidney transplants has not improved to the same extent. Previously it has even shown that higher CsA doses induce more chronic changes in kidney transplants. In the present study our aim was to investigate the effect of CsA on syngenic kidney transplants to explore whether alloimmune response is needed for the development of chronic changes or does CsA itself cause these changes in renal transplants.
Methods: Syngenic kidney transplantations were performed between DA rats. One group of the syngenic grafts were immunosuppressed with CsA. Allografts (DA– > WF) immunosuppressed with CsA were used as controls for the development of chronic changes. CsA doses in both syngenic and allogenic transplantations were adjusted to reach CsA blood trough levels approximately 100 ng/ml. Creatinine and CsA levels were measured once a week. Chronic changes were scored according to Chronic Allograft Damage Index (CADI).
Results: In CsA-treated allografts intense chronic changes were seen, CADI 10.8 ± 0.8. In CsA-treated syngenic grafts marked histopathological changes compared to untreated syngenic grafts were seen, CADI 4.3 ± 0.2 vs. 1.0 ± 0.3. Especially fibrosis and glomerular mesangial matrix accumulation were significantly increased compared to untreated syngenic grafts ($p < 0.05$). In addition, the expression of PDGF, a highly fibrogenic growth factor, was significantly induced in CsA-treated syngenic grafts compared to untreated syngenic grafts. No difference, however, was seen in kidney function between untreated and CsA-treated syngenic grafts based on creatinine levels.
Conclusions: Our results demonstrate that the development of chronic allograft nephropathy may be partly associated with CsA-administration.

W-PO50048

EXPRESSION OF MMP-9 AND ITS ROLE IN THE EARLY STAGE OF CHRONIC ALLOGRAFT NEPHROPATHY IN RATS

HEQUN ZOU[1], YUXIN WANG[1], MIN CHU[2]
Dept. Nephrology, The 5th Hospital of Sun Yat-sen University[1]; Dept. Nephrology, Shenzhen Futian Peoples Hospital, China[2]

Backgrounds: The infiltration of mononuclear cells and the replication and migration of smooth muscle cells (SMCs) from media into the intima in the vascular wall are the cardinal pathological changes in the early stage of chronic allograft nephropathy (CAN). But the mechanism is unclear. We investigated the role of matrix metalloproteinase 9 (MMP-9) and its interaction with TGF-beta1, tubulointerstitial mononuclear cells infiltration and migration of SMCs in the early stage of CAN.
Methods: Kidneys of Fisher (F334) rats were orthotopically transplanted into bilaterally nephrectomized Lewis (LEW) recipients. To suppress an initial episode of acute rejection, rats were briefly treated with cyclosporine (1.5 mg/kg/day) for the first 10 days. Animals were harvested at 12 weeks after transplantation for histological, immunohistochemistry and molecular biological analysis.
Results: The mRNA and protein expressions of MMP-9 were up-regulated in renal tubulointerstitium and vascular wall in the early stage of CAN, when there were interstitial mononuclear cells infiltration and SMCs migration and proliferation, but no proteinuria and no decreased GFR. Moreover the mRNA and protein expressions of MMP-9 were positively correlated with the degree of interstitial mononuclear cells infiltration, the quantity of SMCs in arteriolar wall, and also the increased TGF-beta1 expression in the tubulointerstitium and arteriolar wall.
Conclusion: MMP-9 may play an important role in the mechanism of interstitial infiltration and SMCs migration and proliferation during the earlier period of CAN, and its expression may be induced by TGF-beta1.

W-PO50049

SWELLING OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVEC) UNDER TREATMENT OF CYCLOSPORINE A IN VITRO

UTA HILLEBRAND[1], MARKUS DIETRICH[1], DETLEF LANG[1], MARTIN HAUSBERG[2], HANS OBERLEITHNER[2]
Internal Medicine D[1], Institute of Physiology II[2]

Cardiovascular diseases are the most common cause of death after renal transplantation. They are based on structural and functional changes of the vessel wall, where the endothelium could play a crucial role in the pathophysiological mechanisms. Immunosuppressive drugs like Cyclosporine A (CyA) are known to have negative effects on the vessel wall and so contribute to the development of endothelial dysfunction. Because cell-based mechanisms are yet unclear we inves-

tigated the effects of CyA on single cell volume and apical surface as parameters of endothelial function in human umbilical vein endothelial cells (HUVEC).
HUVEC were grown to confluence under treatment of CyA in dilution series from 10 nM to 5 μM over 3 days. Changes in single cell volume and single apical cell surface were measured using atomic force microscopy (AFM). This technique enables to investigate simultaneously shape, volume and apical cell surface of adherent cells. Both, endothelial cell volume and apical surface was found significantly increased under stimulation of CyA (5 μM CyA: cell volume 3165 ± 79.4 fl/cell, apical surface 1818 ± 58.0 μm^2/cell) compared to non-treated controls (cell volume 1855 ± 36.8 fl/cell, apical surface 1256 ± 23.7 μm^2/cell; $p < 0.01$; n = 40).
We conclude: CyA leads to an intense swelling of endothelial cells with a marked increase of single cell apical surface. This may disturb the barrier function of the endothelial cell layer and thus could be a major step in the development of endothelial dysfunction.

THEME 6: ISSUES IN CLINICAL NEPHROLOGY

Tropical Nephropathies

W-PO60002

RENAL INVOLVEMENT IN 134 CASES OF SNAKE BITE: EXPERIENCE FROM SAUDI ARABIA

AL-HOMRANY MOHAMMED[1], JAFER MALIK[1], ABDULLA SABEEL[1]
King Khalid Uinversity[1]

Snake bite is a common medical problem in southern arabia. In spite of this, no study was conducted to determine the renal involvement following snake bite poisining. This report describes the renal involement in 134 cases of snake bites admitted to Aseer Central Hospital, Southern Saudi Arabia, between 2001–2003. Of 50 snakes killed, 40 were identified as Echis Coloratus and ten as Coluber rhodorachis. All cases were treated with antibiotics and received anti-snake venom. The mean age was 23 ± 17. One hundred cases (74.6%) were male and 34 (25.4%) were females. Seventeen (13%) developed acute renal failure, four cases required hemodialysis. Oliguria was observed in 24% of the cases. Other renal complications include: hematuria 23%, proteinuria 25%, abnormal casts 19%, bilirubinuria 4%, hemoglibinuria 3.1% and glucosuria 3.8%. Late presentaion to hospital, presence of bleeding, jaundice, low platelets, prolonged PT and PTT as well as high SGOT, SGPT and CPK are associated with high risks of developing renal involvement. The prognosis was favourable in all cases except one who died of sepsis. Early hospitalization, effective anti-snake venine and appropriate supportive therapy are needed to prevent serious complications such as renal failure.

W-PO60003

COMPLICATIONS OF MULTIPLE WASP STINGS IN VIETNAM: A CASE SERIES

EUGENE ZELTSER[1], XUAN, BH[2], MAI LE, H[2], NGUYEN, ST[2], RABENOU, RA[1]
New York University[1], Cho Ray Hospital[2]

Introduction: The order *Hymenoptera* includes several groups of stinging insects such as bees, wasps and ants. The anaphylactic effects of single bee stings are widely known among the health professionals. However, we are less aware of the complications suffered by people exposed to the multiple wasp stings.
Patients and Methods: This case series examines the complications of multiple wasp stings among 65 patients presenting to the Cho Ray Hospital (Ho Chi Minh City, Vietnam) over a period of ten years. Patient data was analyzed according to time of presentation to the hospital (early vs. late), and the number of wasp stings (less than fifty stings vs. more than fifty).
Results: Overall, a total of 36 patients (55%) exhibited varying degrees of renal failure, and 31 patients required renal replacement therapy, with an average of 3.9 hemodialysis sessions per patient. The presentation was also complicated by rhabdomyolysis (n = 41), liver function tests abnormalities (n = 31) and hemolysis (as demonstrated by hemoglobinuria in 3 patients). Among the patients requiring renal replacement therapy, twenty-four had evidence of significant rhabdomyolysis suggesting pigment-induced ATN as the cause of renal injury. Total mortality rate was 11.2% (n = 7) with the patients exhibiting persistent hypotension despite treatment with vasopressor therapy, steroids and antibiotics. The number of stings correlated with a more severe presentation: with a higher incidence of mortality, renal failure, hypotension and hemolysis among the patients suffering more than fifty stings.
Conclusion: The constellation of the acute renal failure, hemolysis, rhabdomyolysis, abnormal liver function tests and pressor resistant hypotension is present among the patients exposed to multiple wasp stings. Early recognition of these complications is crucial in the management of the patients with multiple wasp stings. Although most patients recover, mortality rate remains quite significant.

W-PO60004

CLINICAL PROFILE OF PATIENTS WITH ACUTE RENAL FAILURE (ARF): AN INDIAN RAILWAY PERSECTIVE

ABHISHEK BHARGAV[1], R. P. MATHUR[1]
Nephrology, Western Railway Head Quarter Hospital (JRH), Mumbai, India[1]

Aim: ARF continues to be a major cause of mortality and morbidity in hospitalized patients. This study was conducted to know the clinical profile of ARF in a tertiary care hospital of Indian Railways.
Material and Methods: A prospective study of 100 consecutive patients with ARF hospitalized between January 2003 to December 2003 was carried out. All patients underwent a detailed history & relevant investigations pertaining to diagnosis. All patients were treated as per etiology and type of ARF.
Results: The mean age was 53.31 ± 14.9 with 84 males & 16 females. Mean S. Creatinine was 2.95 ± 2.34 mg/dl. 61% had prerenal, 35% had renal & 4% had post renal ARF. 74% had community acquired ARF & 26% had hospital acquired ARF with a mortality of 45% & 42% respectively. 57% were non-oliguric, 37% were oliguric & 6% had anuria. The most common presenting features were fever (47%), vomiting (35%), jaundice (26%), hypotension (23%), loose motions (20%), breathlessness (20%) and hematemesis (12%). The predominant etiological factors were sepsis (23%), chronic liver disease (CLD-20%), acute gastroenteritis (AGE-14%), falciparum malaria (8%), nephrotoxic ARF (8%), cardiac failure (7%), obstructive uropathy (4%), stroke (4%), pneumonia (4%), post operative ARF (3%), leptospirosis (2%), diabetic ketoacidosis (DKA-2%), rhabdomyolysis (1%) and tumor lysis syndrome (1%). Dialysis was required in 15% patients. The overall mortality was 44%. Mortality was highest with sepsis-91% followed by pneumonia -67%, CLD-55%, DKA -50%, falciparum malaria -50%, stroke -50%, post operative ARF-33%, cardiac failure -29% and AGE -7%.
Conclusion: Clinical profile of ARF in a tertiary care center is presented. Majority of cases were non-oliguric. Sepsis was the commonest cause of ARF and was associated with high mortality.

W-PO60005

METHYL ALCOHOL POISONING – ROLE OF ACIDOSIS IN DECISION MAKING

AVADAI AMMAL VIMALA[1], BINU UPENDRAN, JACOB GEORGE, SUSAN UTHUP, MANJU L, MOHANDAS MK[2]
Dept. of Nephrology, Medical College, Trivandrum, India[1], Medical College, Trivandrum, India[2]

Aim: To study the role of acidosis in deciding therapy and prognosis and long term effects on eyes and kidney.
Patients and Methods: Patients admitted with alleged consumption of methyl alcohol in Medical College Hospital, Trivandrum on 10th April, 2003 were studied. Outcome was studied in relation to the amount of alcohol consumed, delay between consumption and initiation of treatment, and severity of acidosis. On admission, arterial blood gas was done in patients with visual impairment, gastric symptoms and tachypnea. All patients were treated with ethanol, B1 vitamins, bicarbonate infusion, methyl prednisolone, and ranitidine. Patients with progressive visual impairment and/or pH < 7.2 were taken up for hemodialysis. Mortality and morbidity were compared in both groups. These patients were followed up after 6 months and a detailed ophthalmological evaluation and tubular function assessment were done.
Results: Twenty eight out of 72 patients with proved methyl alcohol poisoning had pH below 7.35. Mean age was 42 years. Mean delay between consumption and admission was 14 hours. Twelve patients with pH < 7.2 were hemodialysed. Post- dialysis mean pH was 7.3. Duration of hospital stay was 48 hours in dialysis group and 42 hours in non-dialysed group. Mortality was 16.6% in dialysis group versus 6.27% in non-dialysed group. After 6 months, none of the patients

showed evidence of acidification defect. All patients subjectively complained of dimness of vision, but examination did not reveal any abnormality.

Conclusions: Conservative management alone is sufficient in patients with methyl alcohol poisoning with pH > 7.2. Methyl alcohol poisoning did not result in residual renal tubular defects or loss of visual acuity, field defects or fundoscopic abnormality.

W-PO60006

RENAL PAPILLARY NECROSIS, A RARE MANIFESTATION OF VIPER ENVENOMATION WITH RENAL FAILURE

KARUNAN (PRAKASHAN) KANNAMPOYILIL[1], VIMALA AVADIAMMAL[1]
Medical college Trivandrum, Kerala.[1], Nephrology[1]

From 2002 Jan to Dec, 74 snake envenomation cases were admitted, of which 43 were due to viperideae bites. Of these 31 had dialysis requiring renal failure with mortality of 12.5% and one had renal papillary necrosis. This 25 year old manual labourer admitted following viper bite with no past illnesses. He gives no intake of analgesic or other drugs. On admission he had hematuria, oligoanuria and hypotension. He passed bits of fleshy tissue in urine after six hours and sent for histopathologic examination. He received anti snake venom and conservative management. He received hemodialysis to tide over crisis of renal failure, however expired on the fourth day. The histopathology reported as renal papilla with fungal filaments and bacteria. The nucleus of the tubular cells similar to malignant cells. Renal papillary necrosis is rare following viper envenomation. We could not explain the presence of fungal filaments and bacterial clumps in the necrosed tissue and the siginificance of nuclear changes in the tubular cells.

Urinary Tract Infection

W-PO60010

PATHOGENESIS OF ACUTE PYELONEPHRITIS: CLASS II P- FIMBRIAL ADHESIN ENHANCE THE EARLY ESTABLISHMENT OF ESCHERICHIA COLI IN MOUSE KIDNEY

JUNNE-MING SUNG[1], CHIN-CHUNG TSENG[2], MING-CHENG WANG[1], AN-BANG WU[1], JIUNN-JONG WU[3], JENG-JONG HUANG[1]
Departments of Internal Medicine, National Cheng Kung University Hosptial, Tainan, Taiwan[1], Institute of Clinical Medicine, NCKU Medical College[2], Medical Laboratory Science and Biotechnology, NCKU[3]

Acute pyelonephritis (APN) usually results from ascent of organisms from the bladder via the ureter, and *E. coli* is by far the most common infecting pathogen. P-fimbriae have been shown to have a significant association with renal infection, and most pyelonephritogenic *E. coli* in human express the PapG II adhesin. In order to further the role of PapG II adhesin, we selected a pylonephritogenic strain EC114, which possess one copy of *pap* gene and can express the phenotype of PapG II adhesin and Type 1 fimbriae but without the determinants of other virulence factors, including *sfa*, *foc*, *afa*, *cnf1 and hlyA*, for construction of isogenic mutant and compared for colonization ability and histological changes in bladder and kidney in ascending model of BALB/c mice. A strain with *pap*G II mutation, confirmed by PCR and Southern hybridization test, was unable to agglutinate human type A1P1, OP1 RBCs, and was referred to strain MEC114. Female BALB/c mice were challenged transurethrally with 50 μl of low (5×10^4 cfu) or high (5×10^8) dose suspensions of EC114 and MEC114, and assessed on day 1, 3, and 7 after inoculation.

The results showed that the geometric means of quantitative bacterial counts in BALBC/c mice kidney were significantly decreased when challenged with MEC114 than with EC114 on day 3 after inoculation, at both low and high inoculation dose ($P < 0.05$). On the 7th day after inoculation, both the wild type and mutant strains were relatively cleared from the infected kidney.

We concluded that PapG II adhesin can enhance the early establishment of *E. coli* in kidney, but not persistent infection, it might be due to bacterial clearance in immune competent mice.

W-PO60011

ROLES OF HOST AND ESCHERICHIA COLI VIRULENCE FACTORS IN EMPHYSEMATOUS PYELONEPHRITIS

JENG-JONG HUANG[1], CHIN-CHUNG TSENG[2], JIUNN-JONG WU[3], JUNNE-MING SUNG[1], MENG-FU CHENG[1], JYG-YAW HSIEH[4]
Department of Internal Medicine, National Cheng Kung University Hosptial, Tainan, Taiwan[1], Institue of Clinical Medicine, NCKU Medical College, Tainan, Taiwan[2], Department of Medical laboratory Science and Biotechnology, NCKU Medical College[3], Department of Internal Medicine, Yuan's General Hospital, Kaohsiung, Taiwan[4]

Emphysematous pyelonephritis (EPN) is a severe, gas-forming, necrotizing infection of the renal parenchyma and its surroundings. Because of the life-threatening potential and varying mortality rate (7–75%), EPN deserves a special attention. The most common causative organism for EPN is *E. coli* (60–70%). But the precise pathogenesis of EPN remains obscure.

We studied the host and bacterial factors predisposing patients to EPN (n = 47) by comparing them with those for non-EPN (n = 79, including three cases of renal abscess, nine of acute bacterial nephritis, and 67 of acute pyelonephritis). The causative *E. coli* strains were available for 16 of the 47 EPN cases, and for all 79 non-EPN analogs. A total of 14 virulence genes of *E. coli* were detected by polymerase chain reaction (PCR) analysis, including *pap*G I-III, *fimH*, *sfa/foc*, *afa*, *hlyA*, *cnf1*, *iutA*, *iroN*, *iha*, *usp* and *ompT*. The gas volumes produced by *E. coli* were compared between EPN and non-EPN strains cultured in broths at three glucose concentrations (i.e. 100, 180 and 250 mg/dl).

Major urinary tract obstruction was more prevalent in the EPN group. Diabetes mellitus with poor glycemic control (i.e. Hb_{A1C} level >11%) was also more prevalent in the EPN group and was the only host factor independently associated with EPN ($OR = 4.9$; $P = 0.018$). Higher prevalence of the *iroN* and *usp* genes were demonstrated for the EPN strains Multivariate analyses revealed the association between *usp* and EPN with borderline significance ($OR = 8.4$; $P = 0.057$). The gas volumes produced by *E. coli* were significantly increased only when the glucose conc. of broths were elevated to 250 mg/dl in both groups.

In conclusion, diabetes mellitus with poor glycemic control, major urinary tract obstruction and more virulent bacteria are the factors predisposing to EPN.

W-PO60012

A STUDY OF IMPACT OF DELAYED TREATMENT ON RENAL INJURIES IN PEDIATRIC URINARY TRACT INFECTIONS

MOSTAFA SHARIFIAN DORCHEH[1], MARYAM RAJAB NEJAD[2], ABDOLLAH KARIMI[3]
Pediatric Nephrology, Pediatric Infectious Disease Research Center, Mofid Children's Hospital, Iran[1], Pediatric Nephrology, Shaheed Beheshti University of Medical Sciences, IRAN[2], Pediatric Infectious Disease Research Center, Shaheed Beheshti University of Medical Sciences, Iran[3]

Objective: To determine whether the delayed treatment in children with urinary tract infection could predict the risk for renal damage at the time of diagnosis and 6 months after the infection.

Methods: The study population included 100 children aged 1 month to 14 years who admitted with urinary tract infection in Mofid Children's Hospital from March 2000 to September 2004. DMSA renal scan was performed in all patients at the beginning of treatment and 6 month later. All patients had evidence of pyelonephritis and were treated with third generation cephalosporins. Twenty seven patients proceed to perform DMSA Scan 6 months after treatment for evaluation of scars.

Results: Of 100 patients studied, 77 were girls and 23 percent were boys. The mean age was 3.3 years (Range 1 month to 14 years). Organisms responsible for UTI were E.coli in 68%, Streptococcus in 14%, Pseudomona in 10%, Klebsiella in 6% and Candida in 2% of cases.The mean time of delay in treatment was 4.6 days. After 6 months; 51.9 percent of patients who were treated in 2.8 days had normal DMSA Scan and 33.3 percent who were treated after 6 days had renal scarring. The consequence of delayed treatment early in the course of the disease as well as 6 month later is shown in tables 1 and 2.

Table 1 impact of delayed treatment on renal injuries

First DMSA Scan	Mean days delay in Treatment	Number
area in nctionfu cortical reasecDe 1	2.58	25
Decreased cortical function in multiple areas	4.48	31
ed cortical functionGlobal decreas	4.46	13
Cortical defect	6.6	10

Table 2 impact of delayed treatment on renal scars

month 6DMSA after UTI	(Days) Mean delay	Number of patients	Percents
Normal	2.78	14	52
Decreased cortical function	3.3	4	15
Renal scars	6	9	33

Conclusion: Efforts to reduce the incidence and severity of renal scarring should be directed towards rapid diagnosis and effective early management of urinary tract infections in infancy and childhood.

W-PO60014

THE CLINICAL INVESTIGATION OF THE BACTERIA SPECTRUM AND THE ANTIMICROBIAL RESISTANCE IN THE URINARY TRACT INFECTION

PINGYAN SHEN[1], REN HONG[1], CHEN XIAONONG[2], ZHANG WEN[2], LI XIAO[2], CHEN NAN[2]
Department of Nephrology, Rui Jin Hospital, Shanghai Second Medical University Shanghai 200025, China[1], Rui Jin Hospital, Shanghai Second Medical University[2]

Objective: To investigate the bacteria group of urinary tract infection on their distribution characteristics and their sensibility to the antibiotics both in inpatients and outpatients.
Methods: 3332 patients who had the positive results of middle stream urine culture and sensibility test of antibiotics in our hospital from August 2001 to May 2004 were studied.
Results: All the patients got the positive results. 72% of them were gram-negative bacillus, 28% of them were gram-positive coccus. There were obvious differences in the spectrum of bacteria between out-patients and in-patients. Escherichia coli was the most common in out-patients, while pseudomonas aeruginosa was the most common in in-patients. All the clinical departments especially surgical department in our hospital didn't have many differences in the spectrum of bacteria which occur in the urinary tract infection, but pseudomonas aeruginosa and E.facalis occurred frequently in surgical department; Escherichia coli was also the most common in pediatric department and obstetric department. The sensibility tests showed that these bacteria's sensibility to aminoglycosides, third generation cephalosporins, carbapenem antimicrobial agents were up to 75%; Ciprofloxacin had only the sensibility of 37%; vancomycin had the high sensibility to gram-positive coccus of up to 98%.
Conclusion: Escherichia coli is the most common bacteria in the patients with uncomplicated urinary tract infection. The in-patients with often urinary tract infection have basic diseases, in poor general health, complicated with other infections and catheter indwelled and other factors. The infection of gram-negative bacillus and some other conditional bacteria get higher obviously. In the recent years antibiotic-resistance of ciprofloxacin becomes higher obviously. Vancomycin has the large effect on gram-positive bacteria.

Infection Related Nephropathies

W-PO60015

ACUTE RENAL FAILURE IN COMPLICATED FALCIPARUM MALARIA: MANAGED AT OUR INSTITUTE

DIVYESH VIROJA[1]
B. T. Savani Kidney Hospital, Rajkot, India[1]

A prospective study which enrolled 77 patients admitted between April 2003 to December 2004 at our centre. The demographics, clinical presentation, biochemical parameters, requirement for dialysis and outcome were studied.
The mean age (SD) of the patients was 41.27 (18.34) years youngest 7yrs and oldest 85 years [63 (82%) were males and 14 (18%) were females]. 70 (90.9%) patients had fever with rigor, 43 (55.84%) had jaundice & 23 (29.87%) had breathlessness, 47 (61.03%) had oliguria and 58 (75.32%) had haematuria. 21 (27.27%) patients presented with hypotension and 13 (16.88%) with cerebral malaria. On investigations 68 (88.31%) patients had acute renal failure (ARF), 20 (25.97%) had hyperkalaemia, 13 (16.88%) had hypokalaemia, 20 (25.97%) had hypoglycaemia and 46 (59.74%) had proteinurea. Hyperbilirubinaemia was present in 59 (76.62%) and 45 (58.44%) had elevated SGPT. 41 (53.24%) patients had anaemia and 28 (36.36%) had TC >12000. The biochemical and haematological parameters on presentation:

	Mean	S.D.	Minimum	Maximum
Hb	9.83	2.26	5.1	15.1
TC	12231.8	8902	1800	53900
Platelet count	118675.3	45990.62	26000	210000
Bl.urea	126.28	74.63	18	320
S.creatinine	5.48	3.84	0.61	19
S.potassium	4.96	1.62	2.6	9.1
RBS	112.89	81.82	12	384
S.bilirubin	7.47	9.91	0.56	43.5
S.bilirubin (direct)	5.14	6.75	0.35	31.2
SGPT	78.06	107.07	14	622

A large number of patients (26/77) had parasite load >1%, 16 (20.78%) had mixed infection with falciparum and vivex malaria. 40 (51.94%) patients required dialysis 33 haemodialysis and 5 peritoneal dialysis, while 2 patients treated with both. 52 (67.53%) patients had S,creatinine >3 mg/dl, out of them 29 (55.77%) treated with haemodialysis, 5 (9.61%) with peritoneal dialysis and 2 (3.84%) with both. Only 4 patients required haemodialysis even with S.creatinine <3 mg/dl. There were 6 deaths (8%). All the patients treated with artesunate due to chloroquine resistant.
A multiple regression analysis for prognostic factors predicting mortality showed significant association with a high parasitic load (>1%) ($P < 0.03$) Hyperkalaemia ($P < 0.01$), hypoglycaemia ($P < 0.0001$), severe jaundice ($P < 0.05$), cerebral malaria ($P < 0.0001$), total count >12000 ($P < 0.01$), thrombocytopenia ($P < 0.01$). A significant number of patients with falciparum malaria develop renal failure which is associated with hematological abnormalities and evidence of other organ dysfunction. Dialysis is essential to decrease mortality in the patients with ARF.

W-PO60016

RHABDOMYOLYSIS, THE LATE COMPLICATIONS WITH GRAVE OUTCOME IN SARS PATIENTS

HUNG-BIN TSAI[1], JENQ-WEN HUANG[2], JANN-TAY WANG[2], VIN-CENT WU[2], JIUN-LING WANG[2], PO-REN HSUEH[3], TZONG-SHINN, CHU[4], KWAN-DUN WU[5]
Department of Nephrology, St. Martin De Porres Hospital[1], Departments of Internal Medicine[2], Departments of Laboratory Medicine[3], Departments of Primary Care Medicine[4], Departments of Internal Medicine, National Taiwan University Hospital and College of Medicine, Taipei, Taiwan[5]

Background: The purpose of this study is to determine the incidence and clinical impact of rhabdomyolysis in patients of severe acute respiratory syndrome (SARS).
Methods: From March 8 to June 15, 2003, 79 adult patients diagnosed with SARS and treated at a university hospital in Taiwan were studied. Rhabdomyolysis was defined by acute elevation of serum peak creatinine phosphokinase

(CPK) over 1000 U/L, excluding myocardial infarction. Rhabdomyolysis was observed in 9 patients categorized as Group I, the reminders as Group II (n = 70). The clinical characteristics, disease courses, laboratory parameters, and complications were compared between the two groups.
Results: Rhabdomyolysis with peak CPK level occurred 15.1 ± 6.1 days after disease onset. At presentation, Group I patients had higher hematocrit (40.4% vs. 36.4%, $P < 0.05$) and CRP level (7.3 mg/dL vs. 3.7 mg/dL, $P < 0.01$) than Group II. Both the CPK levels at presentation and at the peak were higher in Group I (304.9 U/L vs. 136.4 U/L, $p < 0.05$; and 384065.8 U/L vs. 257.4 U/L, $p < 0.01$, respectively). Group I patients disclosed higher intubation rates for respiratory failure (100% vs. 23.2%, $P < 0.01$), more depolarizing neuromuscular blocking agents use during intubation (77.8% vs. 14.3%, $P < 0.01$), more steroid pulse therapy use (55.6% vs. 8.6%, $P < 0.01$) with earlier intravenous immunoglobulin and steroid initiation day (4.9 day vs. 7.4 day, $P < 0.05$; and 6.0 vs. 8.4 day, $P < 0.01$, respectively). Incidence of acute renal failure (66.7% vs. 10.1%, $P < 0.01$), gastrointestinal bleeding (77.8% vs. 13.2%, $p < 0.01$), bacterial superinfection (66.7% vs. 20%, $P < 0.05$) and mortality rate (66.7% vs. 14.3%, $P < 0.05$) were higher in Group I. The overall case fatality rate was 20.3%. Using multivariate analysis, initial hematocrit and CRP level were two predictors for rhabdomyolysis of SARS patients.
Conclusion: Rhabdomyolysis, a devastating late complication of SARS-CoV infection may be due to immunopathological damage and resulted in acute renal failure. For SARS patients with elevated hematocrit and CRP level at presentation should regularly monitor CPK change.

W-PO60017

OUTCOME IN SEVERE MALARIAL ACUTE RENAL FAILURE

MANOJ RAMSHARAN GUMBER[1], PANDYA J K[1], GUMBER V M[2], THAKAR P A[1], GERA D N[1], TRIVEDI H L[1]
Institute of Kidney Diseases & Research Centre[1], Civil Hospital[2]

Background: Malaria, still a common health problem in India, has a considerable morbidity and mortality. This study reports its occurrence with a serious complication, acute renal failure (ARF), at a tertiary care centre.
Aim of the study: To analyse the clinical profile and determine the outcome, the predictors of outcome in patients of malarial acute renal failure.
Materials and Methods: All patients with ARF who had history and clinical findings suggestive of malaria and malarial parasites on peripheral smears were included in this study. The data on their modes of presentation, etiology, management and outcome have been analysed.
Results: In a retrospective study 120 indoor patients of malarial ARF were studied at IKDRC during the period of January 2004 to December 2004. 92 patients were due to P. Falciparum, 14 of P.Vivax and 14 were of mixed variety. Mean age in the study was 37+/−15.87. Male: Female ratio was 1.3 : 1 and 104 (86.6%) were oligo- or anuric on presentation. Hyperparasitemia was cause of ARF in 34 (28.3%) cases. Mean serum creatinine on admission was 8.34 ± 2.75 mg/dl and 82 (68.3%) patients required renal replacement therapy. 18 (15%) patients were biopsied, 10 (55%) patients had patchy cortical necrosis. Of the cohort, 26 (21.6%) expired, most within 48 h of admission. Of the survivors 74 (78.7%) had complete recovery of renal function while 10 (10.6%) had incomplete recovery and 10 (10.6%) had to be on maintenance haemodialysis. Oliguria, Jaundice, CNS involvement and development of ARDS emerged as bad prognostic factors. There was no difference in Age, Haemoglobin and serum creatinine between the survival and expired group.
Conclusion: In patients who do not succumb early to ARF of severe malaria, treatment with antimalarials and dialysis brings about recovery of renal function.

W-PO60018

MALARIAL ACUTE RENAL FAILURE IN ENDEMIC AREA OF SOUTH INDIA

KRISHNASWAMY SAMPATHKUMAR R[1], MANICKAM SELVAM[1], ASWIN SUBRAMANIAM[1], ARUN PRASANA[1]
Nephrology Meenakshi Mission Hospital and Research Centre, India[1]

Background: Falciparum malaria is well entrenched in a few geographic areas of South India. We present our data on 4 year experience with malarial ARF treated in our hospital.
Aim: 1. To analyse incidence and clinical profile of falciparum malarial ARF. 2. To analyse the risk factors for morbidity and mortality.
Patients and Methods: A retrospective analysis was done from Jan 2000 to Dec 2004. Smear positive falciparum malaria patients with renal involvement were taken up for the study. Their clinical profile and outcome were analysed.
Results: Out of 313 cases of malarial fever 30 were due to falciparum. Of these 18 cases with renal involvement formed the study group. Mean age 51 yrs (r18–75). Male : Female 13 : 5. Presenting features, fever (all-100%), Jaundice (38%), Non oliguric course (83%), Hypotension (22%) and altered sensorium (27%). Anemia (mean HB-8.6 G%), Hyperbilirubinemia (mean Bilirubin 15.4 mg/dl), Renal failure (mean B.Urea 151 mg%, S.Creatinine 4.2 mg%), Low S.Albumin (Mean 19 g/l) were seen. Liver involvement in 38% patients. Metabolic encephalopathy in 27% patients and Respiratory failure in 1 patient. 16 patients were treated with Quinine and 2 patients with artemether. 9 patients were dialysed (50%). IHD in 5, SLED in 2. Two patients were treated with PD. Three patients died (14%), all in the dialysis group. Sudden death was encountered in 2 patients treated with artimether. Requirement for dialysis, Liver cell failure, encephalopathy and artemether therapy were associated with mortality.
Conclusions: 1. 60% of cases admitted with falciparum malaria have renal involvement. 2. Non oliguric ARF is the commonest presentation. 3. Dialysis therapy is required in 50% of cases. 4. The mortality is 18% and is associated with multi organ failure. 5. Use of artimether is associated with sudden death in Malarial ARF.

W-PO60019

RENAL DISEASE IN VISCERAL LEISHMANIASIS

JAI PRAKASH[1], B.KAR[1], S.SINGH[1], SHYAM SUNDER[1], USHA[1]
Department of Nephrology, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005 (India)[1]

Renal involvement in Visceral Leishmaniasis have been reported in the form of proteinuria, microscopic haematuria, acute renal failure and histologic abnormalities in kidney biopsy. However, renal disease in Visceral Leishmaniasis is not widely documented from India, despite Kala-azar being endemic in this country. We have studied incidence and spectrum of clinical renal disease in patients with Visceral Leishmaniasis. Renal tissue under light microscope was studied in six cases. This study included 240 (Male:154 and Female:86) patients with parasitological confirmed diagnosis of Visceral Leishmaniasis over a period of two years (April 2002–April 2004). The presence of oliguria, edema, protienuria, elevated S. creatinine and haematuria either alone or in combination were taken as evidence of clinical renal disease. The renal involvement was documented in 37 (15%) patients. The age (Male:32; Female:03) of the patient ranged between 15–36 years. The spectrum of renal diseases included; proteinuria in the range of 1–2 gm/day (15%), abnormal urinary sediment (4%), edema (9%) and acute renal failure (15%) of cases. Dialytic support was not needed. All patients received Amphotericin B (1.0 mg per kg body wt.) as anti-leishmanial treatment for 15 infusions. Renal histology in six patients revealed; ATN (4), AIN (01) and Thrombotic microangiopathy in (01) patients. We have not observed glomerular lesions in our patient. There was no mortality.
Thus, renal disease can occur during the course of Visceral Leishmaniasis. They were of mild nature and reversible with treatment of Kala-azar without specific treatment. Acute renal failure is mostly related to prerenal factors and overall renal disease carry good prognosis in patient with Visceral Leishmaniasis.

W-PO60020

SEVEN CASES OF HEPATITIS B VIRUS ASSOCIATED NEPHROPATHY WITH NEGATIVE SERUM HBV MARKERS: RECENT DISCOVERIES

XIAYU LI[1], WEI-QIN LIN[1], QIANG HE[1], ZHANG-FEI SHOU[1], HUI-PING WANG[1], JIANG-HUA CHEN[1]
The Kidney Disease Center, The First Affiliated Hospital, Medical College, Zhejiang University, Hangzhou, China[1]

Several studies have discovered that hepatitis B without serum HBV carriage was proved by liver biopsy recently. Now we have paid attention to the incidence of HBV-GN with negative serum HBV markers.
The aim of the present study was to investigate the clinicopathological features of HBV-GN with negative serum HBV makers.
Seven cases of HBV-GN with negative serum HBV was dependent on the presence of an immune complex glomerulonephritis, immunohistochemical localization of HBV antigen and negative serum HBV. Clinicopathological features were assessed and compared with eight of HBV-GN with positive serum HBV makers. The efficacy and safety of immunosuppressive schema such as prednisolone or prednisolone plus mycophenolate mofetil were observed.
Seven cases included six males, one female. Median age was 52 years, which was significant difference from that of patients with positive serum HBV markers. There were no significant difference of urinary protein excretion, creatinine

clearance, and serum levels of albumin, creatinine, alanine aminotransferase between the two groups. In response to immunosuppressive therapy, only one case achieved a partial remission, and the serum alanine aminotransferase level mildly increased in one patients, but serum HBV-DNA of all cases have not increased and serum HBsAg, HBeAg, anti-HBc have maintained negativity.

Except for median age, clinicopathological features were not different between those patients of HBV-GN with and without serum HBV positive. Small-sample and short–term study also suggested that immunosuppressive therapy might have no effect and no tendency of improving HBV replication in treating HBV-GN with negative serum HBV makers.

W-PO60021

HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) IS INVOLVED IN CHRONIC KIDNEY DISEASE (CKD) IN HIV-INFECTED PATIENTS

MINORU ANDO[1], YUUYA NAKAMURA[1], KENJI AZUMA[1]
Tokyo Metropolitan Komagome Hospital[1]

Highly active antiretroviral therapy (HAART) has dramatically improved the course of classic human immunodeficiency virus (HIV)-associated nephropathy (HIVAN) and the prognosis of HIV-infected patients with end-stage renal disease (ESRD). However, HAART is comprised of several nephrotoxic drugs and it has to be continued all through patients' lives. We noted that HAART per se could be related to chronic kidney disease (CKD) in HIV-infected patients. Clinical data including serum creatinine (Cr) levels were monthly examined in HIV-infected patients from the start of HAART at Tokyo Metropolitan Komagome Hospital (Japan). We excluded the data of patients who had had pre-exsisting proteinuria, elevated Cr levels, and clinical history of HIVAN before HAART. The data of 267 patients (245 males and 22 females) were evaluable and abstracted from time of first HIV documentation through 12/31/2004. Prevalence of proteinuria was 14.1%. The number of patients with CKD (doubling of serum creatinine from an initial level <1.0 mg/dl) was nine (3.4%). The longest duration of HAART was 9 years. All CKD patients were male and their Cr levels were 1.2 to 8.7 mg/dl (mean; 2.8 mg/dl). The decrease of 1/Cr was observed first at the time of 2 to 3 years of HAART and became clear in parallel with years of HAART in each CKD patient. Other common complications induced by HAART, such as high blood pressure, glucose intorelance, hyperlipidemia were well treated in all CKD patients. Maintenance hemodialysis therapy was initiated in two patients who had 7 or 9 year experience of HAART. One was treated with a regimen of d4T, ddI, NFV, and the other with a regimen of AZT, 3TC, NFV. **Conclusion:** Our data suggest that a long-term HAART itself induces de novo nephropathy, which may lead to ESRD, in HIV-infected patients.

W-PO60022

CORRELATION OF CD 4 COUNT WITH RENAL DISEASE IN A COHORT OF HIV POSITIVE PATIENTS IN A DEVELOPING NATION

HARI JANAKIRAMAN[1], GEORGI ABRAHAM[1], MILLY MATTHEW[1], SARAH KURUVILLA[1], SUNITHI SOLOMON[2], KUMARASWAMY[2]
SRMC&RI[1], *Y.R.G. Care*[2]

Methods: The study was done over 7 months, HIV positive patients (n-106), male-71, female-35. 101 infected by heterosexual contact, 3-transfusion, and 2-IV drug abuse. Albuminuria was checked by urine dip stick. Renal biopsy was done for patients with proteinuria of ++ or more. Histological examination was by light and electron microscopy.

Results: Proteinuria observed in 31 patients, was correlated (Pearson's correlation coefficient) with CD4 count which showed a negative correlation (P-0.003). Patients with CD4 < 350 have a 3.2 times higher risk of proteinuria than patients with CD4 > 350. Proteinuria did not have any significant relation with the duration from diagnosis. Proteinuria has a negative correlation to the level of hemoglobin (P-0.02).Lower levels of CD4 are associated with lower levels of haemoglobin (P- < 0.0001)

Nine biopsies were done results were, HIVAN – 5, chronic tubulointerstitial nephritis-1, membranous nephropathy-1, one patient with coexistent SLE had proliferative, membranous lupus and also FSGS suggestive of HIVAN and one patient with HCV had diffuse proliferative glomerulo- nephritis.

ARF was in 4% of patients, three patients with a pre renal component and one patient had MODS. Continous ambulatory peritoneal dialysis (CAPD) was done for four patients with HIV and ESRD. Patients were on CAPD for a total of 74 patient months. Three patients on CAPD died at intervals varying from 3–36 months, the other patient continues on peritoneal dialysis for the 13months. There was no increased incidence of peritonitis in these patients.

Conclusion: In our study HIVAN is common (5%) in patients with HIV infection. The incidence of proteinuria in HIV patients is about 26%. Patients with a CD4 count <350 have a higher risk of proteinuria. Proteinuria has a negative correlation to the level of CD4 count and hemoglobin. Lower levels of CD4 counts are associated with lower levels of hemoglobin.

W-PO60023

THE SPECTRUM OF KIDNEY DISEASE IN HIV + PATIENTS IN SOWETO, SOUTH AFRICA

TREVOR E. GERNTHOLTZ[1], STEWART GOETSCH[2], IVOR KATZ[1]
Dumisani Mzamane African Institute of Kidney Disease[1], *University of the Witwatersrand, Johannesburg*[2]

In the United States, HIVassociated nephropathy (HIVAN) is known to be the third leading cause of end stage renal failure in Blacks the 18–64 year old age group. It is a collapsing variant of focal segmental hyalinosis occurring almost exclusively in Blacks. The spectrum of HIV associated kidney disease is not well documented in Africa, where almost three quarters of those who carry this infection reside. We decided to undertake a retrospective review of all the patients with HIV infection who had undergone renal biopsy at our outpatient clinic to try and analyse the patterns of nephropathy prevalent in our patient population.

Method: Chris Hani Baragwanath is a 3000 bed hospital serving Soweto (approximately 2–3 million Black people). Roughly 10–20% of Sowetans are HIV+. Most of our referrals are in patients. There are very few 'cold' GP type referrals because of the poorly developed primary health care structures in the area. We studied all patients who were seen in our out patient department from 1st February 2003 until 31st December 2004 who were HIV+ and who had undergone a renal biopsy. Indications for biopsy were left up to each physician but included non resolving acute renal failure and significant proteinuria. In our institution, HIV testing is performed routinely prior to biopsy.

Results: In our outpatients we had 91 HIV+ patients who were biopsied. 2 were inadequate for diagnostic purposes leaving 89 to study. The results are summarised in the table:

Conclusions: It is clear that there are many different forms of kidney disease occurring in HIV+ patients. HIVAN in fact accounted for less than a third of all cases. Thus it is important to make definite histological diagnoses if we are to eventually understand the aetiology of the various categories and to formulate effective treatments.

W-PO60024

RISK FACTORS FOR DEVELOPING ACUTE RENAL FAILURE IN HIV INFECTED SUBJECTS

NORA FRANCESCHINI[1], SONIA NAPRAVNIK[2], JOSEPH ERON[2], CHARLES VAN DER HORST[2], WILLIAM FINN[1]
UNC Nephrology & Hypertension[1], *UNC Infectious Disease*[2]

Background: Acute renal failure (ARF) is a common cause of renal dysfunction in HIV-infected patients. This study was designed to estimate independent risk factors for developing ARF in ambulatory HIV-infected patients.

Methods: Prospective cohort study of 754 ambulatory HIV-infected patients, older than 17, recruited from a University-based infectious disease clinic in North Carolina between 2000 and 2002. ARF was identified by an algorithm using proportional increases in serum creatinine from baseline renal function, and confirmed by chart review. Clinical and laboratory risk factors were assessed at baseline. Independent risk factors for ARF were estimated by fitting multivariable predictive Poisson regression models.

Results: Mean age was 40 ± 9 years, 68% were male and 61% were black. At enrollment, 31% had a CD4 cell count < 200 cells/mm3, 43% had HIV RNA ≥10000 copies/mL and 10% had a clinical AIDS-defining condition (AIDS). 17% of the patients had hypertension (HTN), 6% had diabetes mellitus (DM), 21% were hepatitis C infected (HCV), and 16% had a history of injection drug use (IDU). The incidence rate of ARF was 5.9 per 100 person-years. Male gender (incidence rate ratio [IRR] = 1.7, P = 0.03), AIDS (IRR = 3.5, P < 0.0001), a CD4 cell count < 200 cells/mm3 (IRR = 2.4, P = 0.001), high HIV RNA levels (IRR = 1.3 for each 1 log10 copies/mL increase, P = 0.006), HCV-infection (IRR = 3.0, P < 0.0001) and DM (IRR = 2.2, P = 0.02) were independently predictive of developing ARF.

Conclusions: Low CD4 count, high HIV RNA levels, a prior clinical AIDS diagnosis, HCV co-infection, DM and male gender are independent risk factors for ARF in ambulatory HIV-infected patients.

W-PO60025

POST-STREPTOCOCCAL ACUTE GLOMERULONEPHRITIS IN BANGLADESHI CHILDREN: CLINICAL PROFILE AND SHORT TERM PROGNOSIS

MD. MUHIBUR RAHMAN[1], SHAMIM AHMED[1], EHTESHAMUL HAQUE[1], MD. NAZRUL NAZRUL ISLAM[1]
Nephrology, Dhaka Medical College, Bangladesh[1]

A total of 97 cases were studied over a period of 2 years after diagnosis of post-streptoccal glomerulonephritis in a renal clinic. The mean age of the patients was 8.35 ± 3.25 years (range 3–15 years). 73 cases were male and 24 cases were female. Commonest mode of presentation were oedema in 96 (98.95%), oliguria in 61 (62.88%), hypertension in 60 (61.85%), gross haematuria in 17 (17.52%), fever in 16 (16.49%), dyspnoea in 11 (11.34%), convulsion in 6 (6.18%) and headache in 5 (5.15%) cases. Fifty six (57.73%) cases had pyoderma and 35 (36.48%) cases had sore throat. In 6 cases no history of sore throat or skin infection was observed. Complications like hypertensive encephalopathy, congestive heart failure and acute renal failure were observed in 7 (7.21%), 5 (5.15%) and 3 (3.09) cases respectively. Investigation showed proteinuria and haematuria in all cases. RBC cast was found in 15 (15.49%) cases. Blood urea and serum creatinine were elevated in 18 (18.21%) and 3 (3.09%) cases respectively. Raised ASO titre was noted in 25 (25.26%) and complement C_3 was low in 46 (47.17%) cases. Follow up study showed complete remission in 78 (80.41%), partial remission in 16 (16.49%) and relapse following remission in 3 (3.09%) cases. It is concluded that post-streptoccal acute glomerulonephritis is still common in Bangladeshi children and short term prognosis is good though certain percentage may go to chronicity.

Calcium-Phosphate Dysregulation and Cardiovascular Disease

W-PO60026

CARDIOVASCULAR DISEASE IN CHRONIC KIDNEY FAILURE – ROLE OF VITAMIN D ANALOGS

J. RUTH WU-WONG[1], MASAKI NAKANE, DOUG DIXON, JUNLI MA[1]
Abbott Laboratories[1]

Vitamin D_3 is modified by vitamin D_3-25-hydroxylase in the liver and 25-hydroxyvitamin D_3-1a-hydroxylase in the kidney to form the active metabolite, 1,25-dihydroxyvitamin D_3. Chronic kidney disease (CKD) patients encounter a much higher risk of cardiovascular disease (CVD) than the general public. The cardiovascular risk factors for CKD patients include conventional and non-conventional factors such as reduced vascular compliance and various hormonal factors. Several vitamin D receptor activators (VDRAs) including paricalcitol and calcitriol are currently available for the treatment of hyperparathyroidism secondary to CKD. Recent clinical data show that vitamin D receptor activators provide survival benefit for CKD patients in the rank order of paricalcitol > calcitriol > no VDRA therapy, independent of PTH and calcium. Moreover, the survival benefit seems to be associated with cardiovascular causes.

In this study, we show that paricalcitol and calcitriol are equally potent in suppressing PAI-1 synthesis and inhibiting cellular proliferation in human coronary artery smooth muscle cells. In As4.1, a JG cell-like cell line derived from kidney tumors in SV40 T-antigen transgenic mice with a high level of renin synthesis, both drugs suppressed renin mRNA expression in a dose dependent manner when the cells were transiently transfected with the human VDR cDNA.

Because of its wider therapeutic window, in the clinical setting paricalcitol is commonly dosed ~3-fold higher than calcitriol to achieve equivalent PTH control without hypercalcemia. Our data are consistent with the clinical observation that paricalcitol at a ~3-fold higher clinical dose than calcitriol results in additional mortality and morbidity benefits in the order of paricalcitiol > calcitriol > no VDRA therapy.

W-PO60027

EFFECT OF HIGH PHOSPHATE ON OSTEOCALCIN MRNA EXPRESSION AND CALCIUM DEPOSITION IN VASCULAR SMOOTH MUSCLE CELLS

XIAO YUN WANG[1], LUWEIPNG[1], ZHAOXIUFEN[1]
Nanjing Medical University[1]

Objective: To investigate tile mechanism of high phosphate-stimulated calcification in vascular smooth muscle cell.
Methods: The cell culturc of bovine aortic smooth muscle was performed. Osteocalein (OC) mRNA expression and calcium deposition in different phosphate concentrations [normal phosphate (Pi 1.5 mmol/L), high phosphate (2.0 mmol/L)] were determined by radioimmunoassay, RT-PCR, and O-cresolphthalein complexone method respectively.
Results: After 72 hours, high phosphate treatment of smooth muscle cells (SMC) enhanced the expression of osteocalcin protein [Pi 2.0 mmol/L versus Pi 1.5 mool/L: (15.03 ± 2.60) pg/μg protein versus (2.98 ± 0.84) pg/μg protein, $P < 0.001$], and the expression of osteocalcin mRNA (OC/GAPDH: 1.91 ± 0.13 versus 0.75 ± 0.04, $P < 0.001$) Hyperphosphate-stimulated calcium deposition in SMC increased significantly in a time-and dose-dependent manner. On day 6, Pi 2.0 mmol/L versus Pi 1.5 mool/L: (77.187 ± 11.692)μg/mg protein versus (25.768 ± 1.750)μg/mg protein, $P < 0.01$. On day 9, (125.399 ± 16.677)μg/mg protein versus (29.046 ± 2.635)μg/mg protein, $P < 0.01$.
Conclusions: High phosphate can directly increase calcium deposition and osteocalcin mRNA expression of SMC. Hyperphosphatemia is an independent factor to stimulate the vascular calcification.

W-PO60028

THE BEHAVIOUR OF THE OSTEOPROTEGERIN / RANKL SYSTEM

DIRK MUELLER-WIEFEL[1], M. WESTPHAL[1], G. HOLST[1], M.J. KEMPER[1]
Univ. Children´s Hospital, Hamburg, Germany[1]

Osteoprotegerin (OPG) and its ligand RANKL (receptor activator of NFK B ligand) play an important role in the regulation of bone resorption. To evaluate the behaviour of this system in uremic children we investigated OPG and RANKL by means of ELISA technique in children with CRF on conservative treatment (CT, n = 33, mean age 7.3 +/–5 yrs) and with ESRD under dialysis (D, n = 26, mean age 10.7 +/–5 yrs) vs 97 controls (C, mean age 7.6 +/–4 yrs). Data were compared with tartrate resistant acid phosphatase (TRAP) and bone alkaline phosphatase (BAP). Median OPG values were 4.2 in C, 4.0 in CT and 4.85 pmol/l in D respectively; median RANKL values were 137 in C, 67 in CT and 26 pg/l in D, respectively. TRAP was decreased in CRF (5.7 vs 8.0 U/l in C) but BAP increased (122 vs 80 U/l in C). Both parameters were unaltered by the degree of renal failure. In C OPG significantly decreased with age ($p < 0.05$). Moreover, under physiological conditions OPG inversely correlated with RANKL ($p < 0.0001$), but not with TRAP or BAP. In CT the inverse correlation of OPG with age ($p < 0.01$ and RANKL ($p < 0.005$) persisted, but got totally lost in D. In both stages of CRF both parameters neither correlated with TRAP nor BAP. The decrease of RANKL with a severitiy of renal failure let to an increase of the OPG/RANKL ratio (0.03 in C, 0.06 in CT und 0.19 in D), thus pointing to a progressive increase of osteoclastic activity with decreasing renal function. Although OPG significantly increased from CT to D ($p < 0.05$), the decrease of RANKL was more pronounced ($p < 0.01$). Thereby RANKL might become a useful sensitive biochemical parameter for the detection of increased bone resorption in CRF children.

W-PO60029

RELATIONSHIP OF LOW SERUM 25 (OH) D3 LEVELS AND THE PREVALENCE OF CARDIOVASCULAR RISK FACTORS BY RACE AND ETHNICITY: DATA FROM NHANES III

DAVID MARTINS[1], MYLES WOLF[2], RAJIV DHAMIJA[1], ASHRAF ZADSHIR[1], NAUREEN TAREEN[1], DEYU PAN[1], BARTON LEVINE[3], KEITH NORRIS[4]
Internal Medicine, Charles R. Drew University, USA[1]*, Harvard School of Medicine*[2]*, West Los Angeles Veterans Affairs Greater Los Angeles Healthcare System and UCLA*[3]*, Charles R. Drew University and UCLA.*[4]

Emerging studies suggest 25 (OH) D3 interacts with the cardiovascular (CV) system. Racial and ethnic minorities have both lower mean serum levels of 25

(OH) D3 and higher rates of CV risk factors and CV disease. Using data from the third National Health and Nutrition Examination Survey (NHANES III) we compared the age-adjusted prevalence of multiple CV risk factors among participants with serum 25 (OH) D3 levels 70 nmol/L (70 nmol/L = recommended normal serum level) across racial and ethnic subgroups (Table).
We conclude the prevalence rate of multiple CV risk factors in the setting of low serum 25 (OH) D3 levels is similar or lower for minority groups in comparison to whites. These data suggest that the increased prevalence of CV risk factors among minorities may be linked to low serum 25 (OH) D3 levels. Further studies are needed to delineate the role of 25 (OH) D3 as an independent factor influencing the prevalence of CV risk.
This project and the authors are supported in part by NIH grants RR03026, RR11145, RR14616, RR019234, and MD00148.

	White	Black	Hispanic
n	3095	3919	3101
Male (%)	41	43	45
Systolic BP. mmHg	125.0	123.0	119.3
SBP > 120 (%)	55.43	49.72	40.70
Diabetes Mellitus (%)	7.09	6.26	6.95
Fasting blood sugar (mg/dL)	101.8	100.3	102.2
Triglyceride (mg/dL)	154.4	110.3	155.2
Triglyceride ≥ 150 (%)	37.2	18.3	36.8
Total Cholesterol (mg/dL)	208.8	196.7	195.7
LDL Cholesterol (mg/dL)	130.9	122.3	118.3

W-PO60030

THE RELATIONSHIP OF 25-VITAMIN D (25-D) LEVELS AND GLOMERULAR FILTRATION RATE (GFR) BY RACE AND ETHNICITY: DATA FROM THE THIRD NATIONAL HEALTH & NUTRITION EXAMINATION SURVEY (NHANES III)

MIQUAEL COTTRELL[1], DAVID MARTINS[1], DEYU PAN[1], SANDRA WILLIAMS[2], KEITH NORRIS[3]
Internal Medicine, Charles R. Drew University – Los Angeles, USA[1]; Mayo Clinic – Jacksonville Fl.[2]; Charles R. Drew University and UCLA – Los Angeles[3]

Several small studies have suggested that many patients with chronic kidney disease (CKD) are deficient in the precursor, 25 (OH) D3. We performed a cross-sectional analysis of 16,992 NHANES III participants not taking 1.25 vitamin D. GFR was assessed using the modified MDRD equation. Patients were designated vitamin D deficient (below 37.5 nmol/L), low normal (37.5–75 nmol/L), and normal (above 75 nmol/L) as suggested by the K-DOQI guidelines. 51% of stage 1 and 2, and 61% of stages 3–5 CKD patients have vitamin D levels below normal. Vitamin D deficient and low normal vitamin D levels (Low D) are highly prevalent particularly in African Americans (AA) compared to Whites (89.4% vs. 53.7%). Mean vitamin D levels for Whites and AA across GFR deciles is shown in the table.
Low D is common in the general population. The prevalence of Low D is higher among whites with lower GFR levels, but is similar among AA, likely due to the overall higher rates of Low D among AA. Low normal and deficient Vitamin D levels should routinely be screened for in both the general and CKD populations.
This project and the authors are supported in part by NIH grants RR03026, RR11145, RR14616, RR019234, and MD00148.

	GFR	<15	15–20	30–40	40–50	50–60	60–70	70–80	80–90	90–95	>95
25-D	White	28.4	56.3	67.7	72.2	70.3	76.8	77.9	82.3	75.2	73.6
25-D	AA	52.0	41.8	54.8	47.1	52.0	52.1	46.6	47.2	47.5	47.7

Others

W-PO60031

THROMBIN TREATMENT ON POSTBIOPSY MASSIVE MACROHEMATURIA

QIN LIN[1]
Division of Nephrology, Fuzhou General Hospital of Nanjing Command, Fuzhou, China[1]

Percutaneous renal biopsy is an invasive procedure that can result in many complications. Although the incidence of massive macrohematuria following kidney biopsy is very low, it is a serious and major complication. In addition to blood transfusion and surgical hemostasis, the conventional anti-hemorrhagic drug is pituitrin. However, its frequent side effects such as hypertension, hyponatremia or acute prerenal failure restrict the usage. Recently, thrombin is commonly used for hemostasis of small vessels, capillary and parenchymatous organ hemorrhage. It could be used repeatedly with caution without notable side effects.
We undertook this study to evaluate the effects of thrombin on the hemostasis of postbiopsy massive macrohematuria. From 2000 to 2003, 1875 percutaneous renal biopsies were performed in our renal units. Among them, 4 patients underwent severe massive macrohematuria following renal biopsy. All these four cases were male, with the age between 28 and 42. They presented continuously large macroscopic hematuria with urocystic clot formation for over 2 weeks. Two patients were treated with pituitrin and blood transfusion; the other remaining two with renal failure were made a trial use of thrombin. A solution of 500 u–1000 u thrombin injected to pelvis locally through right ureter catheter under the urinary ladder scope for 1–2 times, combined with continuous perfusion of urinary bladder to prevent the obstructive nephroureteral clot formation. All the macrohematuria disappeared without primary disease aggravation.
Conclusion: Locally transcatheter use thrombin has a rapid and potent impact on the hemostasis of postbiopsy massive macrohematuria without side effects.

W-PO60032

FUNCTIONAL BLADDER CAPACITY AND RESPONSE TO DESMOPRESSIN (DDAVP) IN MONOSYMPTOMATIC NOCTURNAL ENURESIS

LASZLO KOVACS[1], EVA RADVANSKA[1], MARGARETA GECÍKOVÁ[1]
Department Of Pediatrics, University Children S Hospital, Bratislava, Slovakia[1]

The objective of this study was to correlate the estimated daytime functional bladder capacity with response to DDAVP in patients with monosymptomatic nocturnal enuresis.
Patients and Methods: 46 children with at least 3 enuretic episodes per week were included. Micturition volumes were measured and the maximal daytime functional bladder capacity was determined as the largest voided volume during ad lib fluid intake and, on a separate day, following a standard oral water load (20 ml/kg over a period of 30 minutes). Values were also expressed as a percent of predicted age-adjusted bladder capacity based on formula – patient age +2 × 30 = cc. DDAVP treatment was given for 2-weeks (20 ug intranasally at evening), in non-responders (with less than 50% reduction of wet nights as compared to baseline), the treatment was continued with adding oxybutynin to DDAVP for an additional 2-week period.
Results: 30 of the 46 patients (65%) were desmopressin-responders, while the remaining 16 patients were classified as nonresponders (reduction of the weekly frequency of wet nights from 4.73 ± 0.53 to 0.67 ± 0.16, $p < 0.001$ and from 6.87 ± 0.12 to 4.37 ± 0.32, $p < 0.05$, respectively). In addition to fewer baseline wet nights per week ($p < 0.05$), responsiveness DDAVP alone was also associated with older age (12.00 ± 0.81 versus 9.75 ± 0.71, $p < 0.05$), and larger age-adjusted functional bladder capacity after a standard water load (402.3 ± 52.5 versus 213.7 ± 21.1, $p < 0.001$). In non-responders, the combined DDAVP plus oxybutynin treatment resulted in an additional reduction in the number of wet nights per week (from 4.37 ± 0.32 on DDAVP to 2.37 ± 0.49 on combined treatment, $p < 0.05$).
Conclusions: Responsiveness of enuretic children to DDAVP is adversely affected by reduced functional bladder capacity. These results have implications regarding the potential use of combination of DDAVP and an anticholinergic for enuretics with lower functional bladder capacity who are not responding to DDAVP.

W-PO60033

COMPLICATED HEMOLYTIC UREMIC SYNDROME IN AN ADULT PATIENT-CASE REPORT

LUMINITA C. ARDELEAN[1], VIORICA BUMBEA[1],
OANA CONSTANTINESCU[1]
Nephrology and Dialysis, Emergency Clinical Hospital, Romania[1]

Hemolytic-uremic syndrome (HUS) is defined as a combination of microangiopathic hemolytic anemia with variable degrees of thrombocytopenia and renal failure.
A 33-year-old male was referred to our hospital for acute renal failure (ARF) developed after a 2 weeks episode of hemorrhagic diarrhea. On admission he presented fever, abdominal pain and oliguria. Laboratory tests revealed leucocytosis 14700/mmc, anemia 8.3 g/dl, thrombocytopenia 54000/mmc, LDH 1819 UI/l, CRP 4.1 mg/dl, urea 180 mg/dl, creatinine 7.3 mg/dl, bilirubine 2 mg/dl. Blood smear showed 3–4 schistocyte/field, anisocytosis, reticulocytes 5.6%. Hepatitis B, C, HIV serology negative. Blood, stool and urine culture were negative. Imunological tests were negative for vasculitis, systemic lupus eritematosus.
Abdominal ultrasound revealed enlarged kidneys, distended small bowel (mostly fluid), peritoneal fluid between loops. Colonoscopy and rectal biopsies ruled out inflammatory bowel disease.
Clinical and laboratory data supported the diagnosis of HUS with ARF and the patient was started on plasmapheresis (P) (4 sessions) interspread with continuous veno-venous hemodiafiltration (CVVHDF)(6 sessions). After 10 days of P and CVVHDF the renal function slowly improved, urine output increased to 1000 ml/day and azotemia decreased. After a few days of clinical improvement the patient had severe abdominal pain; clinical and radiological data (CT scan and plain abdominal X-ray) suggested acute abdomen. He was referred to surgery, a large bowel perforation was discovered and partially colectomy was performed (Hartmann procedure). The patient continued to improve in intensive care unit without any renal replacement procedures.
We presented a rare adult case of probably idiopathic HUS with ARF, with renal improvement after P and CVVHDF. In spite of the favorable outcome of pathogenic treatment, the patient developed a serious complication-microangiopathic ischemic colitis with large bowel perforation.