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Abstracts

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INTERACTIONS BETWEEN THE RAS, ADVANCED GLYCATION AND NF-kB IN THE DIABETIC KIDNEY: INTERVENTIONAL STUDIES

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Recently it has been suggested that there are interactions between the advanced glycation pathways and the renin-angiotensin system (RAS), possibly via effects on oxidative stress and intracellular signalling pathways including NF-kB and protein kinase C. This study examined interactions in experimental diabetic nephropathy.

Male Sprague Dawley rats injected with streptozocin (55 mg/kg). Three groups of diabetics were studied for 12 weeks (n = 6) and were randomised to no treatment, aminoguanidine (AG), an inhibitor of AGE formation at 1 g/L in drinking water (DAG) or an ACE inhibitor, ramipril (RAM) at 1 mg/kg/day (DRAM). Control groups (untreated, AG and RAM) were studied concurently (n = 6). Assessment of glycaemic control (HbA1c), albuminuria (AER) and immunostaining for AGE, the AGE receptor RAGE and the activated p50 subunit of NF-kB were performed in all groups.

	HbA1c (%)	AER (mg/24 hrs)	RAGE	AGEs
Control	3.6 ± 0.2*	0.5 ×/1.2*	7.1 ± 0.7*	2.2 ± 1.1*
DIAB	10.6 ± 0.4#	1.0 ×/1.2#	18.3 ± 4.6#	11.7 ± 2.0#
DRAM	9.1 ± 0.7*#	0.6 ×/1.2*	15.8 ± 2.5#	1.5 ± 0.8*
DAG	9.7 ± 0.5*#	0.9 ×/1.3*	12.5 ± 0.5#	2.3 ± 0.6*
CRAM	3.3 ± 0.6*	0.5 ×/1.2*	6.1 ± 1.9*	2.3 ± 0.7*
CAG	3.9 ± 0.2*	0.4 ×/1.4*	6.5 ± 1.5*	3.5 ± 1.9*

*$p < 0.05$ vs DIAB group. #$p < 0.05$ vs Control group. Mean ± SEM or geometric mean x/tolerance factors shown.

Reductions in AGE immunostaining (table 1) were demonstrated with both AG and RAM. There was increased staining for the p50 subunit of NF-kB in the diabetic kidney which was attenuated by both RAM and AG treatment. the increases in RAGE immunostaining was not normalized by either drug in diabetic rats. These findings suggest cross-talk between the renin-angiotensin system and advanced glycation in diabetes possibly via the transcription factor, NF-kB.

2

LOVASTATIN DOWNREGULATES RENAL MYOFIBROBLAST FUNCTION *IN VITRO*

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Interstitial fibrosis is recognised as the best histological predictor of progressive renal disease. Myofibroblasts contribute to this process through several functions including hyperproliferation, collagen and collagenase synthesis and reorganisation of extracellular matrix. Recent limited *in vitro* studies suggest that 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase inhibitors may reduce renal injury not only through their lipid-lowering effects but also by antagonising myofibroblast function. This study therefore examined the effects of lovastatin on interstitial myofibroblast behaviour *in vitro*. Primary cultures of rat renal cortical myofibroblasts were grown by explantation and characterised by immunohistochemistry. Dose response effects of lovastatin (0, 15, 30 μM) in DMEM & 10% FCS were examined on 1) myofibroblast kinetics (tritiated thymidine incorporation and serum-stimulated cell growth), 2) total collagen synthesis (trititated hydroxyproline incorporation), 3) collagenase synthesis, 4) collagen I lattice contraction and 5) actin filament rearrangement. Lovastatin decreased myofibroblast proliferation (51.0 ± 4.6% of control levels at 30 μM; $p < 0.05$) and growth (55.6 ± 16% of control levels at 15 μM; $p < 0.05$). Likewise, lovastatin decreased collagen production (14.4 ± 4.2% of control levels at 30 μM; $p < 0.01$). Synthesis of collagenases was decreased in the presence of lovastatin. Similarly, collagen I lattice contraction was inhibited when lovastatin was added at 30 μM ($p < 0.05$). Actin filament rearrangement was partially disrupted following incubation with 30 μM lovastatin, a mechanism which may contribute to inhibition of lattice contraction. Downregulation of myofibroblast function by HMG CoA reductase inhibitors may confer direct benefits in the treatment of progressive renal scarring.

3

ANGIOTENSIN II MODULATES DECREASED ANTIOXIDANT EXPRESSION AND REACTIVE NITROGEN SPECIES PRODUCTION IN EXPERIMENTAL HYDRONEPHROSIS

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To evaluate the importance of angiotensin II (AII) in mediation of reactive nitrogen species-induced cell injury and antioxidant dysfunction, male Sprague-Dawley rats underwent unilateral ureteral obstruction (UUO) with/without candesartan (1 mg/kg bwt.), NG-nitro-L-arginine methyl ester (L-NAME; 100 mg/kg) or aminoguanidine (100 mg/kg) administration. At 24, 48, 96 hours and 1 week after UUO, Northern blot analysis demonstrated a restored pattern of Cu-Zn superoxide dismutase (SOD; 122.7 + 14.0 vs. 97.0 + 8.8, $P < 0.021$, n = 4) and catalase (168.8 + 38.2 vs. 66.0 + 7.35, $P < 0.029$, n = 4) mRNA expression when rats were administered the angiotensin II type 1 receptor (AT1) or NO inhibitors. Western blot analysis revealed candesartan pretreatment significantly down-regulated nitrotyrosine protein levels (164.3 + 32.0 vs. 26.3 + 18.9, $P < 0.003$, n = 3), a marker of peroxynitrite production, and decreased iNOS protein expression (152.3 + 35.0 vs. 41.0 + 29.2, $P < 0.013$, n = 4). Immunohistochemistry localized inducible NO synthase (iNOS) protein to the dilated proximal tubule epithelial cells (PTCs), collecting ducts, and medullary thick ascending limbs of the obstructed kidneys. Nitrotyrosine was found in areas of interstitial expansion and dilated PTCs of the obstructed kidney by 1 week after UUO. In vitro studies demonstrated that AII-mediated down-regulation of catalase mRNA expression in cultured rat PTCs was prevented following candesartan pretreatment. These results demonstrate a central role for AII-mediated reactive nitrogen species production and antioxidant enzyme down-regulation in the renal cortex of UUO rats and in in vitro studies using cultured rat PTCs.

4

p53Pro72Arg POLYMORPHISM IS ASSOCIATED WITH ALBUMINURIA AMONG ABORIGINAL AUSTRALIANS

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Renal and cardiovascular disease is common in Aboriginal communities in Australia. As part of broader studies in a remote NT Aboriginal community, we examined the associations of the Pro → Arg polymorphism in codon 72 of the p53 gene (previously associated with cardiovascular disease in a Caucasian population) with renal & cardiovascular disease. Serum, urine (spot albumin/creatinine ratio—ACR) and DNA samples for 222 adult subjects (age 17–80, mean 39 years, 96 male) in the community were available for the current study.

The Arg/Pro mutation at codon 72 was determined using PCR-RFLP (the CCC → CGC transition has created a restriction site for BstUI). The wild-type PP homozygote has a single 182 bp band, mutant AA homozygote 133 bp and 49 bp bands, and the AP heterozygote has 182 bp, 133 bp and 49 bp bands.

P allele frequency was 0.55 [0.50–0.60]. Genotype distribution was in Hardy-Weinberg equilibrium ($p = 0.5$). ACR rose in a P allele dependent manner (PP > AP > AA, $p = 0.01$) There was no significant variation in calculated GFR, age, sex, smoking or blood pressure across groups. HbA1c measurements increased significantly with presence of P allele ($p = 0.03$). The relationship between p53 genotypes and ACR was partly independent of HbA1c (adjusted $p = 0.05$). A significant interaction between tobacco smoking and p53 was seen; increasing ACR with presence of P allele was only seen in smokers ($p = 0.002$ for difference with non-smokers). This interaction remained after adjustment for HbA1c.

Genotype (n)	Geom. Mean ACR (g/mol)	HbA1c (median and IQR, %)
AA (45)	2.1 [1.2–3.4]	5.6 [5.1–6.2]
AP (97)	3.7 [2.5–5.4]	5.8 [5.4–6.4]
PP (61)	5.1 [3.2–8.2]	6.0 [5.5–7.0]

Our study for the first time demonstrates a significant relationship between albuminuria and a p53 gene variant, which may also be modified by smoking.

5

TARGET GENES OF THE WILMS' TUMOUR SUPPRESSOR GENE AS IDENTIFIED BY cDNA EXPRESSION PROFILING

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Mutations in the Wilms' tumour suppressor gene, *WT1*, occur in Wilms' tumour (WT) and in the Denys Drash syndrome (DDS) of infantile nephropathy, XY pseudohermaphroditism, and WT. *WT1* encodes multiple nuclear proteins, all with four C-terminal zinc finger motifs. WT1 proteins can both activate and repress putative target genes *in vitro*, although the *in vivo* relevance of these putative target genes is unclear. To validate these target genes and search for new WT1 targets, we have established two cell based systems; (i) stable transfectants of the mouse mesonephric cell line, M15, expressing a common DDS mutant form of WT1 and (ii) human embryonal kidney (HEK293) cells which inducibly express individual WT1 isoforms. A comparison of the expression profiles of M15 and DDS transfectants was performed using Clontech 588 gene arrays and a custom nylon array of potential WT1 target genes (http://www.cmcb.uq.edu.au/pubs/array.html.). *Wnt-4*, a member of the Wnt gene family of secreted glycoproteins, was downregulated in C2A. *Wnt4* is critical for the mesenchyme-epithelial transition during kidney development, making it an attractive putative WT1 target. We have mapped human *Wnt-4* gene to chromosome 1p35–36, a region of frequent LOH in WT and have characterized the genomic structure of the human Wnt-4 gene. Several potential WT1 binding sites exist within the first 1.2 kb of the immediate promoter which are being investigated for regulation by WT1. WT1 inducible HEK293 cells have been expression profiled over a 24 hour time course using a human microarray chip containing 19 200 human transcripts. Novel genes showing significant induction are being investigated for their expression pattern during kidney development using in situ hybridisation of both kidney sections and wholemounts of kidney explant cultures. In both systems examined there is little evidence to support the vaildity of most previously described WT1 target genes.

6

TRANSFORMING GROWTH FACTOR-β1 (TGF-β1) GENE EXPRESSION AND ACTIVATION IN THE PATHOGENESIS OF FIBROSIS IN PROTEINURIC RENAL DISEASE IN HUMANS

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TGF-β is secreted in a latent form requiring extra-cellular modification to become biologically active. Therefore, gene expression may not equate with biological activity. βig-H3 is a uniquely TGF-β1 responsive gene product used to assess TGF-β1 biological activity in tissue specimens. In this study, two contrasting renal diseases were examined, both with nephrotic syndrome, but only one with progressive fibrosis and renal impairment. Using competitive RT-PCR, TGF-β1 mRNA was quantitated in human biopsy samples from focal segmental glomerulosclerosis (FSGS) (n=11), and minimal change disease (MCD) (n=6) and compared with normal nephrectomy samples (n=4). Immunohistochemistry was performed using anti-βig-H3 antibody, and analysed by quantitative image analysis.

	FSGS	MCD	Nephrectomy
TGF-β1; copy no./ngm RNA (95th centile confidence interval)	12456 (8690, 17854)*	58140 (19341,17555)*	774 (498, 1202)
βig-H3; % area (±sem) glomerulus, tubulo-interstitium	4.7(±1.0)**, 6.9(±0.9)%**	0.4(±0.1), 1.4(±0.2)%	1.3(±0.4), 0.6(±0.2)%

TGF-β1 gene expression was significantly greater in both FSGS and MCD compared to nephrectomy*(p0.0001). However, βig-H3 protein expression was increased only in FSGS **. This study demonstrates increased gene expression of TGF-β1 in both FSGS and MCD. However, biologically active TGF-β1 was only identified in FSGS, where progressive fibrosis and decline in renal function are a feature. These findings suggest that extracellular activation of TGF-β1 may be a key determinant in the fibrotic response to proteinuric renal disease.

7

LONG TERM OUTCOMES OF STENT INSERTION FOR RENAL ARTERY STENOSIS

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Renal artery stenosis is a common condition frequently treated with percutaneous angioplasty and stent insertion. The outcomes of this procedure, and the subgroups of patients particularly likely to derive benefit, have not yet been clearly defined. We retrospectively examined the records of 198 consecutive patients undergoing stent insertion at our institution, and obtained adequate follow up information on 148 (75%), in whom a total of 182 renal arteries had been treated. The median duration of follow up was 23.2 months. Technical success was achieved in 144 patients (97%). Complications occurred in 19 patients (13.3%), with major complications occurring in 10 (7.0%) including 1 death. A fall in average systolic blood pressure of 13.2 mmHg (12.1–14.3 mmHg) was seen, with a fall in diastolic blood pressure of 10.1 mmHg (9.3–10.9 mmHg) without a significant change in the number of antihypertensive drugs used. A rise in serum creatinine was seen in 53 patients (36%) while 12 patients (8.1%) had a sustained fall. Survival rates were 82% at 2 years and 71% at 5 years. Dialysis free survival rates of 78 and 57% were observed over the same intervals. Baseline creatinine above 0.15 mmol/L (Relative Risk 8.1, p=0.0003) and age over 70 (Relative Risk 4.2, p=0.0002) were strong predictors of worse survival on multivariate analysis. When dialysis free survival was considered, the presence of another disease affecting the kidney also predicted worse outcome (relative risk 2.7, p=0.004), while the use of angiotensin converting enzyme inhibitors was protective (relative risk 0.47, p=0.04). Insertion of renal artery stents is a generally safe and effective treatment for hypertension associated with renal artery stenosis. The effects of the procedure on renal function are less clear. Patients with elevated baseline creatinine, advanced age and another renal disease have an increased incidence of death or dialysis, while the use of ACE inhibitors after stent insertion is protective.

8

DISADVANTAGE AND VARIATION IN THE INCIDENCE OF ESRD IN AUSTRALIAN CAPITAL CITIES

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We examined variation in the incidence of ESRD in capital cities (CC) to explore the relation between socioeconomic disadvantage and the incidence of ESRD. We conducted a retrospective analysis using ANZDATA for 5013 patients from CC who started ESRD treatment between 01 April 1993 and 31 Dec 1998. We used the postcode at the start of treatment to assign patients to CC regions using concordances provided by the Australian Bureau of Statistics (ABS). We used 1996 Census place of usual residence counts to calculate standardised incidence ratios with 95% confidence intervals for each region. Rates for the total Australian resident population were used as the reference. The ABS has developed indexes to describe the socio-economic characteristics of an area. This study uses the Index of Relative Socio-Economic Disadvantage (IRSD), derived from the 1996 Census. 1000 equals the mean value for all Australia and low IRSD values indicate more disadvantaged areas. We calculated Pearson correlation coefficients to determine the association between the ISRD values and the standardised incidence ratios for ESRD. The standardised incidence ratio for ESRD within CC varied from 0.37 to 3.23. There was marked variation within most CC. Mapping the standardised incidence ratios reveals geographic sectors of CC that have an excess of ESRD in population terms. There was a significant correlation (r=–0.41, p=0.003) between the standardised incidence ratio for ESRD and the IRSD. If the relatively disadvantaged CC areas (IRSD<1000) had the same adjusted incidence rate of ESRD as the relatively advantaged CC areas (IRSD>1000), 463 ESRD cases (22.8% of those in the disadvantaged areas) would be avoided. CC areas that are more disadvantaged have a higher incidence of ESRD. Socio-economic factors may be important determinants of the risk of developing ESRD.

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ASSOCIATION OF RENAL SIZE AND P53PRO72ARG POLYMORPHISM IN AN ABORIGINAL POPULATION WITH HIGH RATES OF RENAL FAILURE

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The relationship of the cell growth regulator gene p53 and renal size was investigated in a remote coastal Aboriginal community. A total of 177 people (95 M : 82 females): mean age 28.2 years, range 10.9 to 64.3) had DNA testing and a renal ultrasound. Kidney length, width and depth were measured, kidney volume was calculated (KV = KL × KD × KW × 0.523) and then corrected for body surface area (corrKV) to provide a measure independent of age, height and weight. PCR-RFLP method was used for the Arg/Pro mutation at codon 72.

Table 1: *Characteristics of the study population by the p53 genotype.*

P53	No.	Age (yrs)	BMI (kg/m^2)	Mean KV (cm^3)	corrKV (cm^3/m^2)
AA	31	24.5 (±11.4)	20.8 (±4.3)	111.8 (±25.0)	144.5 (±29.4)
AP	96	28.0 (±14.2)	21.9 (±4.7)	116.6 (±33.5)	144.0 (±34.5)
PP	50	30.4 (±13.9)	23.2 (±6.2)	159.3 (±43.8)	159.3 (±35.4)

Kidney volumes increased with increasing body size (mean KV), and this difference is evident even after correction. The AA homozygotes had the smallest kidneys and the PP homozygote the largest kidneys (ANOVA p = 0.045).

CorrKV was larger in those with a PP allelle compared with the other allelles [corkv = 159.3 (±45.4) vrs 144.1 (±33.2)]; ttest, p = 0.0345). When the analysis was restricted to those over 18 years of age, the difference became more marked [168.3 (±51.9) vs 141.9 (±32.6) p = 0.0095]

It appears that the PP allele is associated with renal enlargement. If this association holds true, it may be that the PP allelle drives or facilitates nephron hypertrophy and therefore renal enlargement. While nephron enlargement is an essential element of renal growth during childhood and adolescence, excessive hypertrophy of individual nephrons (glomeruli) may result in premature glomerulosclerosis and predispose to albuminuria and renal failure.

11

A CLINICAL TRIAL IN PATIENTS WITH OVERT TYPE 2 DIABETIC NEPHROPATHY

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The purpose of this trial was to determine whether the angiotensin II receptor blocker irbesartan or the calcium channel blocker amlodipine would provide renal protection from the progression of overt type 2 diabetic nephropathy. 1715 patients with hypertension and type 2 diabetic nephropathy were randomized to daily treatment with 300 mg irbesartan, 10 mg amlodipine, or placebo in a prospective double masked trial. Blood pressure control was to the same target < 135/85 mmHg in all groups. Primary end point was a composite of time to doubling of entry serum creatinine, development of end stage renal disease (ESRD) or all cause mortality. Time to a secondary composite cardiovascular end point was also measured. The mean patient follow-up was 2.6 yr. Treatment with irbesartan was associated with a 20% risk reduction of the primary composite end point events when compared to placebo (p = 0.024) and a 23% risk reduction versus amlodipine (p = 0.006); There was a 33% risk reduction with respect to doubling of serum creatinine favoring irbesartan when compared to placebo (p = 0.003), and a 37% reduction versus amlodipine (p ≤ 0.001). A 23% risk reduction of ESRD for irbesartan relative to both placebo (p = 0.074) and to amlodipine (p = 0.074) was observed. The effect size of the risk reduction was not changed by differences in achieved blood pressures. Serum creatinine for the entire cohort rose 24% more slowly (p = 0.008) in patients assigned to irbesartan than in placebo and 21% more slowly than amlodipine (p = 0.02). Proteinuria was significantly reduced in the irbesartan group throughout the study and not in the amlodipine or placebo groups. There were no significant differences in the risk of all cause mortality or in the cardiovascular composite end point. We conclude that the angiotensin II receptor blocker irbesartan is an effective protective agent against the progression of type 2 diabetic nephropathy. This renoprotection is independent of blood pressure reduction.

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SUSTAINED REDUCTION IN NATURAL DEATHS AND RENAL FAILURE AT FOUR YEARS FROM A SYSTEMATIC RENAL AND CARDIOVASCULAR (CV) TREATMENT PROGRAM IN AN ABORIGINAL COMMUNITY

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In Nov 1995 we started a systematic renal and cardiovascular treatment program in an Aboriginal community with a 3–5-fold increase in mortality and a peak ESRD incidence of 2700 pm. Treatment was centered around use of perindopril (Coversyl, Servier), and rigorous BP control (initial goal < 130/85, later < 125/75), and attempts to control glucose and lipids. Eligible were all people with BP ≥ 140/90, diabetics with urinary albumin/creatinine ratio (ACR, gm/mol) 3.4+ (microalbuminuria threshold) and all people with overt albuminuria (ACR 34+). We previously reported a 55% reduction in natural deaths and renal failure in the treatment group at a mean follow up of 2.1 yr. We now report results through June 30, 2001, with maximum follow-up up to 4.8 years and a mean of 3.4 years. 269 people have been enrolled, or 30% of all adults. Rates of terminal endpoints in the treatment group were compared with of historical controls matched for disease severity. 41 controls and 24 people in the treatment group reached a terminal event. Survival was better in all clinical groups of the treatment cohort, but most marked in the 65% of people who had baseline ACR 34+. In these, 4 year survival was 94.3% in treated group vs 85.8% in controls. All categories of terminal events were reduced by ≥ 45%, after adjustment for age, sex and ACR, as shown by the hazard ratios (CI) below for the 'Intention to Treat' group vs Controls. Survival was enhanced if people with baseline GFR < 30 were excluded.

	all cause deaths	renal death	nonrenal death	CV death
all	0.50 (0.3–0.9)	0.49 (0.2–1.1)	0.53 (0.3–0.9)	0.55 (0.2–1.3)
w/GFR > 30	0.40 (0.2–0.8)	0.31 (0.1–0.9)	0.41 (0.2–0.8)	0.43 (0.2–1.2)

Treatment was more effective in nondiabetics than diabetics and in people with hypertension (≥ 140/90) than those without hypertension. Sustained reductions in natural deaths and renal failure in the community as a whole, compared with their pretreatment peaks, support these estimates. Cost savings, based on avoidance of dialysis alone, are many millions. Programs like this are enormously effective, and should be offered all Aboriginal communities in need as a matter of urgency.

12

ANTI-NEUTROPHIL CYTOPLASM ANTIBODIES IN PATIENTS WITH ANTIBODIES TO THE GLOMERULAR BASEMENT MEMBRANE: INCIDENCE AND CLINICAL FEATURES

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The effect on patient outcomes of co-existing anti-glomerular basement membrane (GBM) antibodies and anti-neutrophil cytoplasm antibodies (ANCA) is not clear. We retrospectively assessed all patients with anti-GBM antibodies presenting to the Statewide Renal Service over the past ten years (n = 82). ANCA positive and negative patients differed significantly at presentation (Table 1).

Variable	ANCA negative (n = 59,72%)	ANCA positive (n = 23, 28%)	P value
Age (years)	50.6 (sem 1.8)	72.6 (sem 1.1)	< 0.001
Smoking	34%	74%	0.001
Systemic symptoms	9%	35%	< 0.001
Time to presentation (days)	7.1 (sem 0.6)	12.5 (sem 2.3)	0.002
Creatinine (μmol/L)	567 (sem 42)	956 (sem 52)	< 0.001

Patients with both antibodies were more likely to require dialysis (n = 21, p < 0.001), and to die (n = 19, p < 0.001). Those with ANCA had significantly more (p = 0.002) crescents on renal biopsy (84%; sem 2 vs 68%; sem 3). In logistic regression, ANCA predicted ESRF independent of age and sex (OR 12.3, CL 1.10, 141.0) and also predicted mortality (OR 2.5, CL 1.6, 4.27). Creatinine was significantly related to ANCA (b = 161.7, se 77.4, p = 0.04) and number of crescents (b = 6.7, se 1.4, p < 0.001) independent of age and sex. Our finding of poorer outcomes in patients with co-existing ANCA and anti-GBM antibodies may influence management decisions.

P13

TIMING OF SOX18 EXPRESSION DURING KIDNEY DEVELOPMENT

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The vascular endothelial growth factors (VEGF-A, B, C and D) and their receptors, including flk-1 and flt-1, are critical for the processes fo vasculogenesis and angiogenesis during development and postnatally. Components of this cascade have also been shown to be elevated in renal carcinoma (RCC) and regulation of this pathway may be valuable in the control of RCC[1]. Other components of this pathway are also being elucidated, including the transcription factors Sox18[2]. This nuclear DNA-binding protein is related to the sex-determining gene, Sry, due to the presence of a Sry-like HMG box. Sox19 expression has been investigated in both flt-1 –/– and flk1 –/– mice, with a loss of Sox18 expression in the latter only. We aimed to characterise the onset and pattern of experession of Sox18 during the development of the vasculature of the mouse kidney. Expression was assessed using wholemount and section in situ hybridisation of murine kidney explant cultures. Sox18 was compared to VEGF-A, VEGF-B, flk-1, flt-1, flt-4, Tie-1 and ephrin B2 expression over a four day period with explants removed at either 11.5 dpc (pre glomerulogenesis) or 12.5 dpc (early MET). 11.5 dpc kidneys appeared to undergo vasculogenesis in culture, as previously reported[3], with Sox18expression being very similar to that seen for flk-1 although coming on slightly later. This supports the suggestion that Sox18 follows flk-1 expression and spatially documents the expression of many other markers in this pathway during kidney development.

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P14

INTERLEUKIN-1β (IL-1β) STIMULATES HUMAN RENAL CORTICAL FIBROBLAST (CF) DNA SYNTHESIS AND EXTRACELLULAR MATRIX PRODUCTION THROUGH A TRANSFORMING GROWTH FACTOR-BETA (TGFβ) DEPENDENT MECHANISM

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One of the hallmarks of progressive renal disease is the development of tubulointerstitial fibrosis. This is frequently preceded by macrophage infiltration, raising the possibility, that macrophages relay fibrogenic signals to resident tubulointerstitial cells. The aim of this study was to investigate the potential fibrogenic role of IL-1β, a macrophage-derived inflammatory cytokine on CF. Primary cultures of human CF were established and incubated for 24 h in the presence or absence of IL-1β (1 ng/ml). IL-1β significantly stimulated DNA synthesis (356.7 ± 39% of control $p < 0.003$), fibronectin secretion (261.85 ± 11% of control, $p < 0.002$), cell associated fibronectin accumulation, and nitric oxide (NO) production (337.7 ± 34.5% of control, $p < 0.002$). TGFβ (1 ng/ml) and the phorbol ester, phorbol 12-myristate 13-acetate (25 nM), produced similar fibrogenic effects to IL-1β. Neither a NO synthase inhibitor (N^G-methyl-L-arginine, 1 mM) or a protein kinase C (PKC) inhibitor (bisindolylmaleimide 1, 0.5 μM) altered the enhanced level of fibronectin secretion or DNA synthesis seen with IL-1β treatment. However, addition of a TGFβ neutralising antibody significantly reduced IL-1β induced fibronectin secretion (IL-1β, 278 ± 66% vs IL-1β + αTGFβ 157.1 ± 22%, $p < 0.02$), and totally abrogated IL-1β induced DNA synthesis (293 ± 58% vs 92.9 ± 11%, $p < 0.005$). In conclusion, IL-1β significantly stimulates CF DNA synthesis, NO release and fibronectin production. The fibrogenic and proliferative action of IL-1β on CF appears not to involve activation of PKC or production of NO, but is at least partly TGFβ dependent.

P15

INSULIN-LIKE GROWTH FACTOR(IGF)-I STIMULATES IN VITRO HUMAN RENAL CELL CARCINOMA (RCC) GROWTH

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IGF-I is a potent proximal tubule cell (PTC) mitogen, which has been implicated in the progression of a number of human cancers. Previous work in our laboratory on paired normal and malignant human renal tissues has suggested that IGF-I and several of its binding proteins (IGFBP-3 and -6) are upregulated in clear cell RCC. To further elucidate the role of IGF-I in RCC growth, we established primary cultures of normal and malignant PTC from patients undergoing radical nephrectomy for clear cell RCC. The RCC cells demonstrated evidence of cytologic atypia and uniformly stained positively for both vimentin and cytokeratin. Incubation with exogenous IGF-I (100 ng/ml) for 24 hours markedly stimulated thymidine incorporation by RCC compared with normal PTC (365 ± 85% vs 120 ± 7% of controls, respectively, $p < 0.01$). The IGF-I analogue, LR^3-IGF-I, (which binds the IGF-I receptor but not IGFBPs), stimulated RCC DNA synthesis less potently than an equivalent concentration of IGF-I (100 ng/ml; 186 ± 42% controls vs 363% ± 85% of controls, respectively, $p < 0.01$). Exposure of RCC cells to neutralising anti-IGF-I-receptor antibody did not significantly alter cell growth (109 ± 7 of controls, p = NS). RCC cells incubated for 24 hours with human renal cortical fibroblast conditioned media (containing 3.9 ± 0.5 ng/ml endogenous IGF-I) did not exhibit significant stimulation of thymidine incorporation (111 ± 11% of controls, p = NS). In conclusion, IGF-I exerts a greater stimulatory effect on the growth of human RCC than compared with normal PTC. The enhanced mitogenic action of IGF-I on RCC is at least partly due to augmentation by ambient IGFBPs. No significant autocrine or stromal-epithelial paracrine IGF-I pathway was identified as a possible mechanism for facilitation of *in vivo* RCC growth.

P16

NITRIC OXIDE (NO) OPPOSES ANGIOTENSIN II (ANG II)—FACILITATED AUTOREGULATION (AR) IN THE INTACT KIDNEY

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The role of AngII and NO in renal autoregulationwere investigated in intact kidneys from Sprague-Dawley rats perfused at 37°C with a modified Krebs-Henseleit biocarbonate buffer. AngII, and phenylephrine (PE) were directly infused into the renal artery. In AR studies (n = 6), pressure was ramped by 10 mmHg intervals over the range 70 to 110 mmHg. The pressure was then lowered and the ramp repeated first in the presence of infused AngII or PE, then with vasoconstictor plus 50 nM methacholine (Mch) and finally with 0.1 mM papaverine alone. Experiments were also performed in the presence of 10 μM L-NAME, and L-NAME plus 1 μM sodium nitroprusside (SNP). AngII and PE elicited dose-dependent decreased in flow and increases in pressure. There was a linear relationship between pressure and flow under control conditions. In the presence of AngII greater than 50 pM, an AR plateau was present, and the autoregulation index (ARI) was 0.11 ± 0/07, –0.01 ± 0/06, –0.13 ± 0.22 respectively for 75, 100 and 200 pM AngII ($p < 0.01$ vs conrol). Infusion of Mch antagonised AR, increasing the ARI respectively to 0.44, 0.46 and 0.44. Papaverine completely abolished AR and increased the ARI above the control level from 0.58 ± 0.04 to 1.4 ± 0.09. No AR was observed in the presence of PE. Indomethacin lowered the AR-facilitating threshold concentration of AngII. L-NAME produced AR under control conditions (ARI = –0.27 ± 0.06, $p < 0.001$) and this effect was overcome by infusion of SNP (ARI = 0.93 ± 0.07). The isolated perfused rat kidney is a powerful model for studying AR and endothelial function in the absence of neural and endocrine factors. The results suggest the AR is inhibitied under control conditions by NO and vasodilator prostaglandins and can be increased by AngII but not PE.

P17

ISCHEMIA-REPERFUSION INJURY AND K_{ATP} CHANNELS

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ATP-dependent K^+ channels (K_{ATP}) are present in the basolateral membrane of proximal tubules where they account for the majority of the cation conductance and are responsible for recylcing of K^+ which enters the cell via Na, K-ATPase. They are also present on the mitochondrial inner membrane where opeing of K_{ATP} preserves mitochondrial viability and matrix volume during ischemia. The role of K_{ATP} channel modulation in renal ischaemia reperfusion injury (RIR) was investigated using an isolated perfused rat kidney model. IRI was induced by 2 by five minute periods of warm ischemia, followed by 45 minutes of reperfusion. Rnela function parameters were compared between the different groups. IRI significantly decreased urine to perfusate ^{14}C-insulin ration (U/P), glomerular filtration rate (GFR) and increased fractional excretion of sodium (FENa) and potassium (FEK) ($p<0.01$) when compared to non-ischaemic controls. Pre-treatment with 200 µM diazoxide, a K_{ATP} opener, reduced the post-ischaemic increase in FENa ($P<0.05$). A combination of diazoxide plus 10 µM glibenclamide, a K_{ATP} channel blocker, significantly inhibited the diazoxide effect on post ischaemic FENa ($p<0.01$). Pre-treatment of kidneys with idazoxide had no effect on post ischaemic renal vascular resistance (RVR), urine flow rate (UFR), U/P, GFR, and FEK. Glibenclamide significantly decreased post ischaemic RVR and UFR, but had no significant effect on other renal function parameters. Other than a significant increase in RVR produced by glibenclamide, diazoxide and glibenclamide had no effect on non-ischaemic control kidney function. Our results suggest that K_{ATP} channel activation has a protective effect and that bloade has an injury enhancing effect on renal epithelial cell function during IRI. This may be mediated through K_{ATP} channel modulation on the cell and/or mitochondrial inner membrane.

P18

PROTEIN AND AMINO ACID METABOLISM IN UREMIC AND NONUREMIC ACIDOSIS

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The pathogenesis of accelerated catabolism of proteins in renal insufficiency is not clear. The aim of the present study was to evaluate the effect of acidosis.

Changes in protein and amino acid metabolism were estimated using L-[1–14C]leucine in rats with acidosis induced by infusion of 0.2 M HCI and in acute renal failure induced by bilateral nephrectomy (BNX). Controls consisted from saline infused and sham operated animals.

In rats with acidosis induced by HCI infusion we observed a marked increase in plasma amino acid concentration associated with increased whole-body proteolysis and leucine oxidation. BNX caused significant decrease in plasma amino acid levels, a decrease in whole-body protein breakdown, protein synthesis, and in leucine clearance. In addition, a marked increase in leucine oxidised fraction caused by the decreased rate of leucine incorporation in body proteins was observed in BNX rats.

It is concluded that (1) acidosis and activated branched-chain amino acid (valine, leucine and isoleucine) oxidation are an important factor in protein wasting development and (2) changes in protein and amino acid metabolism in BNX rats are remarkably different to those observed in HCI infused rats. The cause of protein than in protein synthesis, while in BNX rats a grater decrease in protein synthesis than in proteolysis was observed.

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P19

NA^+ TRANSPORT AND GROWTH IN HUMAN PROXIMAL TUBULAR CELLS (PTC) EXPOSED TO ALBUMIN

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The progression of renal disease correlates strongly with both hypertension and the degree of proteinuria. As hypertension in proteinuric renal disease is primarily volume-dependent, this study aimed to determine the effects of albumin on PTC growth and sodium transport. Primary cultures of human PTC in serum free medium (5 mM glucose) were exposed to delipidated bovine serum albumin (0.1 or 1.0 mg/ml) for 48 hours. Cell growth was assessed using cell number, total cell protein and 3H-thymidine incorporation. NHE3 activity was determined by measuring the EIPA-sensitive component of 22Na uptake. Results are expressed as mean ± SEM as a % of the control (no albumin) values. A polyclonal antibody to human NHE3 produced in our laboratory was used to determine the changes in distribution of NHE3 following albumin exposure. Exposure of hPTC to 0.1 mg/ml albumin had no effect on cell growth parameters, but significantly increased Na+ uptake to 111.2 ± 4.8% ($P<0.05$). This increase was due to an increase in NHE3 activity of 118.0 ± 6.7% ($P<0.05$). In contrast, cells grown in 1.0 mg/ml had a pronounced increase in cell number to 149.6 ± 11.1% ($P<0.0001$) with concomitant decreases in the level of protein per cell (77.0 ± 5.8%; $P<0.005$) and 3H-thymidine incorporation per cell (65.8 ± 4.2%; $P<0.0001$). 22 Na uptake remained unchanged from control levels. FITC-albumin uptake confirmed that primary cultures of hPTC endocytosed albumin in a dose and time dependent manner. Confocal analysis revealed that 0.1 mg/ml albumin resulted in an apparent increase in NHE3 in the perinuclear region that was reversed at 1.0 mg/ml albumin. These results suggest albumin uptake by the human PTC is associated with enhanced NHE3 activity. We postulate that the increase in NHE3 levels facilitates albumin uptake, but if dysregulated in renal disease, proteinuria, abnormalities in cell growth and hypertension may result.

P20

HIGH GLUCOSE UPREGULATES PLASMINOGEN ACTIVATOR INHIBITOR-1 (PAI-1) IN HUMAN PROXIMAL TUBULAR CELLS (PTCS) AND CORTICAL FIBROBLASTS (CFS)

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The plasmin-dependent pathway has been recognised as an important regulator of extracellular matrix turnover in the kidney. Overexpression of PAI-1 has been reported in experimental models of progressive renal disease. The current study aimed to define the effect of elevations in extracellular glucose concentrations on PAI-1 expression in human PTCs and CF.

Primary cultures of PTCs and CF were grown to confluence and then exposed to either 5 mM or 25 mM D-glucose for 72 hours. Total RNA was extracted and transcribed into cDNA. Competitive RT-PCR assay was used to detect changes in the expression levels of PAI-1 mRNA. Western blots were subsequently performed on supernatant to confirm whether changes in expression were reflected in PAI-1 protein secretion.

PAI-1 message in both cultured human PTCs and CF was confirmed by RT-PCR and subsequent sequencing of the PCR products. Exposure to 25 mM glucose resulted in a 184 ± 33% (mean ± SEM; $P<0.05$) increase in the level of PAI-1 in CF; and 200 ± 30% ($P=0.08$) increase in PTCs when compared to cells grown in 5 mM glucose. Exposure to 25 mM glucose had no effect on the levels of the housekeeping gene b-actin. Absolute PAI-1 expression level in CF varied from $9.27\times10{-}4$ fM to $319.73\times10{-}4$ fM, with a clear and reproducible inter-patient variability. The production of PAI-1 protein by Western blot analysis reflected the changes observed by competitive RT-PCR.

The results demonstrate that high glucose increases PAI-1 expression in both PTCs and CF. Excessive inhibition of the plasmin-dependent pathway may play an important role in the renal interstitial fibrosis observed in diabetic nephropathy. The relevance of the differences in resting levels of PAI-1 and those induced by exposure to high glucose in the predisposition of certain patients to diabetic nephropathy remains to be determined.

P21

CHARACTERISATION AND INVESTIGATION OF PARAQUAT TRANSPORT AND TOXICITY IN A HUMAN PROXIMAL TUBULAR CELL LINE HK-2: APPARENT LACK OF CATIONIC TRANSPORT SYSTEM

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The proximal tubular cell line HK-2 was characterised and used to investigate transport and toxicity of paraquat (PQ) a cationic herbicide. Characterisation by electron microscopy revealed cellular features consistent with proximal tubular metabolically active cells. Enzymatic activity of brush border enzymes alkaline phosphatase (ALP) and gamma glutamyltransferase (γGT) were moderate in the HK-2 cells when compared to high activity in the positive control porcine proximal tubular (LLC-PK_1) cells and low activity in the negative control canine distal tubular cells (MDCK). The lysosomal enzyme acid phosphatase (AP) showed high activity in the HK-2 with comparison to moderate activity in the LLC-PK_1 and no activity in the MDCK. The HK-2 cells expressed negligible factor 8 antigen using human foetal fibroblasts as a positive reference. HK-2 cells did not transport PQ, a divalent cation; nor Tetraethylammonium (TEA) a monovalent cation. TEA was transported by the positive control LLC-PK_1 cells. Flux studies were terminated due to paracellular leakage of mannitol through the HK-2 epithelia. Measurements of cell viability after exposure to PQ showed that HK-2 cells were initially resistant to the toxic effects of PQ when compared to the positive control LLC-PK_1 cells. There was no significant time or dose dependant effect of PQ on HK-2 cells in 2, 6 or 24 hour incubations at any PQ concentration (0.001 mM to 1.5 mM PQ). 72 hours post exposure to PQ, cellular viability was unchanged in the 2 hour but significantly decreased at 6 and 24 hour exposures. This contrasted with the LLC-PK_1 which showed time-dose dependant toxicity initially and then the capacity to regenerate in 72 hours. Although HK-2 cells showed morphologic and enzymatic characteristics consistent with proximal tubular cells, they did not transport PQ or TEA. These two cations have been previously shown to be actively transported into proximal tubular cells. The HK-2 cells have been immortalised by transduction with a human papilloma virus and it may be as a result of this process that the cationic transport system has been deleted or modified.

P22

THE RELATIONSHIP OF VON WILLEBRAND FACTOR TO CORONARY HEART DISEASE AND CONVENTIONAL RISK FACTORS. A PRELIMINARY STUDY ON THE ROLE OF ENDOTHELIAL DYSFUNCTION ON THE INCIDENCE OF CORONARY HEART DISEASE IN DIABETIC NEPHROPATHY PATIENTS

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Atherosclerosis is multifactorial process and often associated with risk factors such as dyslipidemia, hypertension, diabetes mellitus and smoking habit. A high incidence of Coronary Heart Disease (CHD) has been reported. Plaque rupture and thrombus formation have been identified as the most common mechanisms. The close association between von Willebrand factor (vWf) and thrombus formation or atherogenesis suggests that high vWf level may be an useful indirect indicator. This is a preliminary study to assess the role of endothelial dysfunction on the incidence CHD in diabetic nephropathy. Fasting plasma vWf, total cholesterol, LDL cholesterol, HDL cholesterol, fasting glucose, blood pressure and smoking habit were recorded in 80 male Old Myocardial Infarction (OMI) (40–70 years) in cardiovascular sub unit Dr Hasan Sadikin General Hospital and in 80 normal controls were paired according to sex and age. A significant higher difference was observed for systolic blood pressure ($p=0.042$), total cholesterol ($p=0.007$), HDL cholesterol ($p=0.01$) and fasting glucose level ($p=0.001$) in OMI patients. Smokers and ex-smokers have an CHD odds ratio of 1.93. OMI patients have higher levels of vWf compared with that of controls (145.9% vs. 121.4%, $p=0.01$). Patients with vWf concentration in the upper quartile (>159%) had an CHD adjusted odds ratio of 2.4. vWf was correlated with total cholesterol ($r=0.178$, $p=0.02$), LDL cholesterol ($r=0.168$, $p=0.03$), blood sugar ($r=0{,}203$, $p=0{,}005$) and diabetes ($r=0.185$, $p=0.01$). From multiple regression analysis it is found that diabetes is associated with vWf ($p=0.018$). In conclusion vWF is an independent risk factor for CHD in this study.

P23

ISOLATION OF HUMAN GLOMERULAR AND TUBULOINTERSTITIAL ENDOTHELIAL CELLS BY IMMUNOMAGNETIC SEPARATION

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Abnormalities in the structure and function of glomerular endothelial cells are well recognised to play a pivotal role in the development of progressive renal disease. However the vascular abnormalities observed in the tubulointerstitium correlate more strongly with renal prognosis. Hence the isolation and culture of microvascular endothelial cells from the glomerulus and tubulointerstitium of the human kidney is critical to the investigation of both glomerulopathies and renal tubulointerstitial disease.

We have developed a simple and reproducible method for the isolation of both human tubulointerstitial and glomerular endothelial cells using immunomagnetic separation with anti-PECAM Dyna beads. The cells obtained by this method were characterised morphologically and immunohistochemically. The cells had a characteristic cobblestone appearance and Wiebel Palade bodies were present. The cells also stained positive for von Willebrand Factor, PECAM and thrombomodulin and negatively for antifibroblast surface antigen and anti a-smooth muscle actin. Importantly, differential staining patterns were observed with von Willebrand Factor in glomerular and tubulointerstitial cells.

The cells cultured were a homogenous population as contamination by mesangial and fibroblast cells was avoided by manual weeding at an early stage and by reducing the requirement for human serum from 20% to 10%. These cells have been cultured through a number early passages without any significant changes in their morphological characteristics.

In summary, we have developed a unique and novel method for the isolation of both glomerular and tubulointerstitial endothelial cells. It is significant that these cell types clearly have different characteristics suggesting specific functional roles. The application of these model cell systems will be invaluable in the investigation of disease processes in these discrete areas within the kidney.

P24

ATORVASTATIN'S ROLE ON TGFB1 PRODUCTION BY HUMAN ENDOTHELIAL CELLS

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The ubiquitous cytokine TGFB1 is involved in both physiological and pathophysiological responses. However the circumstances in which it exerts a beneficial compared to a deleterious effect in the presence of disease has not been delineated. It has been shown to enhance atherosclerotic plaque stabilisation through its antiproliferative effects on the vascular smooth muscle cell. Since statins are of proven benefit in plaque stabilisation and hyperglycaemia is a known risk factor for atherosclerotic progression, we measured the effects of atorvastatin on levels of TGFB1 on human endothelial cells released under normal and high glucose conditions. Total TGFB1 production was measured using an ELISA assay on human umbilical vein endothelial cell supernatant. As Big H3* is a TGFB1 inducible protein, active TGFB1 was measured indirectly by performing western blots measuring BigH3 on cell lysates.

Exposure of 25 mM glucose over 72 hours was associated with a significant release of total TGFB1 ($162.1+7.8\%$; $p<0.0001$) and a significant upregulation in Big H3 ($155.6+5.7\%$; $p<0.001$). 1 mmol/L atorvastatin in 25 mM glucose induced a similar increase in total TGFB1 release ($173+9.7$; $p<0.0001$); however the levels of Big H3 induced significantly exceeded that induced by 25 mM glucose alone ($206.4+12.4\%$; $p<0.0001$). In the absence of high glucose, 1 mmol/L atorvastatin exposure was associated with both an increase in total TGFB1 ($133.5+6.4\%$; $p<0.001$) and Big H3 ($158.5+10.1\%$; $p<0.0001$).

In summary, the increase in TGFB1 released from endothelial cells in high glucose conditions suggests a beneficial effect is conferred on plaque stabilisation in patients with diabetes mellitus. This beneficial response is amplified in the presence of statins independent of ambient glucose levels.

* Big H3 was kindly donated by A/Prof R Gilbert, St Vincent's Hospital, Fitzroy.

P25

ROLE OF $CD4^+CD25^+$ T CELLS IN THE ALLOIMMUNE RESPONSES

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A subset of $CD4^+$ T cells that express the IL-2 receptor alpha chain (CD25) have been identified as the regulatory cells that maintain tolerance to transplanted tissue and auto-antigens. We compared the *in vitro* and *in vivo* re-activity of naïve $CD4^+CD25^+$ and $CD4^+CD25^-$ subsets to unfractionated $CD4^+$ T cells from DA rats and their response to allogeneic strains. In MLC, proliferation of $CD4^+CD25^+$ T cells to allogeneic dendritic cells was not significantly different against self-dendritic cells and had markedly less proliferation compared to $CD4^+$ and $CD4^+CD25^-$ T cells. $CD4^+CD25^-$ cells had a greater response than that of unfractionated $CD4^+$ cells against allogeneic cells. It was thought the $CD4^+CD25^+$ cells might regulate the $CD4^+CD25^-$ in an unfractionated $CD4^+$ population. After separation of $CD4^+$ into the two subpopulations, we re-combined them in differing ratios, $CD4^+CD25^+$ cells suppressed proliferation of $CD4^+CD25^-$ cells. The higher the ratio of $CD4^+CD25^+$ cells, the greater the suppression. RT-PCR showed similar induction of Th1 (IL-2, IFN-γ) and Th2 (IL-4, IL-5, IL-10) cytokines in both $CD4^+$ and $CD4^+CD25^-$ sub-populations in response to allogeneic strains. $CD4^+CD25^+$ cells responding to alloantigen had no cytokine mRNA induced. *In vivo*, there was a marked difference in capacity of subsets to restore PVG cardiac allograft rejection in irradiated DA hosts, $CD4^+CD25^-$ cells were faster (8–10 d) than unfractionated $CD4^+$ cells (11–14 d) ($p < 0.001$) and $CD4^+CD25^+$ had no capacity to restore rejection (>100 d). Mixing $CD4^+CD25^+$ with $CD4^+CD25^-$ delayed rejection time (11–16 d) compared to that with $CD4^+CD25^-$ alone ($p < 0.001$). These studies showed $CD4^+CD25^-$ T cells were the principal mediators of rejection and MLC proliferation. In unfractionated $CD4^+$ T cells, $CD4^+CD25^+$ regulate the $CD4^+CD25^-$ cells but themselves have little capacity to proliferate in MLC or effect rejection. These results suggest naïve $CD4^+CD25^+$ T cells have a non-specific regulatory response on $CD4^+CD25^-$ T cells and may play a role in induction of transplant tolerance.

P26

FACTOR H-RELATED PROTEIN-5 IN HUMAN RENAL BIOPSIES

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Factor H-related protein 5 (FHR-5) is a newly described human plasma protein with structural similarities to Human Factor H & 4 other related proteins. Unlike the other FHRs, FHR-5, was initially identified at the protein, rather than cDNA, level in human glomeruli. Preliminary data suggest that FHR-5 has complement (C) regulatory properties similar to Factor H. A prospective study of 100 consecutive biopsies was performed, using the anti-FHR-5 monoclonal antibody and indirect immunofluorescence. The pattern of FHR-5 deposition is very similar to that of other C components, C3 and SC5b–9. It is detected in all C containing glomerular immune deposits and has a pattern of extraglomerular deposition which is also similar to that of other C components.

Table—Incidence of glomerular FHR-5 deposition in renal biopsies	No of cases	Incidence of detection C3	SC5b-9	FHR-5
Thin basement membrane nephropathy	12	0	0	0
Minimal change disease	4	0	0	0
Focal glomerular sclerosis	8	2	6	5
Membranous nephropathy	10	10	10	10
Membranoproliferative GN type 1	1	1	1	1
IgA nephropathy	20	19	18	20
Post infectious glomerulonephritis	2	2	2	2
Lupus nephritis	7	7	6	7
ANCA associated GN	6	1	1	0
Diabetic nephropathy	2	1	2	2
Hypertensive nephrosclerosis	2	0	1	1
Interstitial nephritis	3	0	1	0
Transplant, interstitial rejection	4	1	1	1
Transplant, no rejection	11	1	2	1
Total	92			

This study shows FHR-5 to be strongly associated with glomerular C deposition and suggests a role in C activation or regulation. It may be an important regulator of glomerular disease.

P27

INTRINSIC RENAL CELL DERIVED TUMOUR NECROSIS FACTOR-α IS A MAJOR CONTRIBUTOR TO MURINE CRESCENTIC GLOMERULONEPHRITIS

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Crescentic anti-GBM glomerulonephritis (GN) is attenuated in TNF-α deficient mice, whereas administration of TNF amplifies GN. The contribution of inflammatory cell and intrinsic renal cell derived TNF-α to the development of crescentic GN was studied in TNF chimeric mice created by transplantation of bone marrow (BM) from TNF-α deficient mice (TNF-α–/–) into wildtype (WT) mice and vice versa. GN was induced by an i.v. dose of sheep anti-mouse GBM globulin and injury was assessed 21 days later.

	WT	TNF-α BM +/+	TNF-α BM –/–	TNF-α–/–
Crescents (%glom)	22.3 ± 1.4	14.3 ± 1.9*	20.5 ± 2.0	13.0 ± 1.7†
Proteinuria (mg/24 h)	9.6 ± 1.4	1.6 ± 0.2‡	3.3 ± 0.3‡	2.6 ± 0.5‡
Creatinine (μmol/L)	25.8 ± 1.9	17.7 ± 1.3*	21.6 ± 1.4	15.2 ± 0.8†

* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ c.f. WT.

Chimeric mice with absent intrinsic renal cell derived TNF-α (TNF-α BM +/+) showed similar protection to mice with total TNF-α deficiency. However chimeric mice lacking TNF-α derived from BM cells (TNF-α BM–/–) showed less protection against development of crescentic GN. These studies demonstrate that intrinsic renal cell derived TNF-α is required for full expression of crescentic GN. They suggest that intrinsic renal cells are the major source of the TNF-α which contributes to inflammatory injury in crecentic GN.

P28

THE EFFECT OF pH AND NUCLEOPHILES ON COMPLEMENT ACTIVATION BY HUMAN PROXIMAL TUBULAR EPITHELIAL CELLS

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Activation of urinary complement proteins in situ by proximal tubular epithelial cells (PTEC) may contribute to the mediation of tubulointerstitial injury in patients with significant proteinuria. However, the mechanism involved is unclear, and the role of changes in urinary pH and in the concentrations of urea or ammonia requires further clarification.

The protein fraction of urine samples from 9 patients with proteinuria >1.5 g/day was purified. A cell Elisa involving cultured HK-2 PTEC was used to investigate the capacity of urinary protein to promote the deposition of both C3 and C9 on the cell surface. The effect of variations in pH [5.5–8.0] and in the concentration of urea and ammonia was also examined. C3 was purified and used to further investigate the mechanism of complement deposition.

Urine samples from the majority of patients induced deposition of C3 and C9 on the surface of HK-2 cells via the alternative pathway. This process was maximal at acidic pH values. Preincubation of urinary complement or serum with urea or ammonia inhibited C3 deposition. Purified C3 incubated with HK-2 cells showed no evidence of activation in the absence of other complement components.

These data suggest that bicarbonate protects against complement-mediated tubular damage by increasing the local pH, rather than by inhibiting the generation of ammonia. PTEC appear to activate complement through provision of a 'protected site' on their surface, rather than by the activation of C3 by convertase-like protease[s].

P29

EFFECT OF A HIGH PROTEIN DIET *IN UTERO* ON KIDNEY DEVELOPMENT

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Fetal exposure to a maternal low protein diet leads to reduced nephron endowment which is linked to increased risk of hypertension/renal disease later in life. This study investigated the hypothesis that a high protein diet (HPD) fed to pregnant rats would result in high birth weight offspring with supernumerary nephron endowment in their kidneys.

Female Wistar-Kyoto rats were fed either a high (54% protein) or a normal (20%) protein diet (NPD) throughout pregnancy. Male birth-weight-matched pairs of rats from the same litter were used. The rats were perfusion fixed at 4 weeks of age (nephrogenesis in the rat is complete by postnatal day 10), and kidneys were processed for stereological estimation of glomerular number (a measure of efficiency of nephrogenesis) using the physical disector/fractionator method. Total length and surface area of glomerular capillaries and total renal filtration surface area were also stereologically determined.

Offspring of rats fed the HPD during pregnancy were not significantly different in birth weight to offspring of rats fed the NPD (4.26 ± 0.45 g and 4.22 ± 0.50 g, respectively). Kidney development did not appear to be altered by the dietary treatments, with no significant differences between HPD and NPD offspring in kidney weight (0.337 ± 0.090 g and 0.369 ± 0.016 g, respectively) or glomerular number (26 700 ± 4730 and 27 000 ± 3360 glomeruli/kidney, respectively). Similarly, there were no significant differences in total length or surface area of glomerular capillaries or total renal filtration surface area (1720 ± 460 and 1450 ± 351 mm^2 respectively).

In conclusion, HPD exposure during fetal life does not lead to kidneys with supernumerary nephrons in rats.

P31

ACCURACY OF PD ADEQUEST COMPUTER BASED KINETIC MODELLING TO ACHIEVE ADEQUATE DIALYSIS

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There is increasing evidence in the peritoneal dialysis population that KT/V correlates with patient survival. The current ISPD guidelines recommend a KT/V > 2.09. We have 123 PD patients and all patients have a PET and Adequest within 4–8 weeks of commencing dialysis. On the PET the membrane transport types are: 55%HA, 37%LA, 5%H, 3%L. The initial Adequest documented that 49% achieved adequate dialysis (KT/V > 2.09) and 51% did not, 12% 2–2.09, 13% 1.8–199, 26% < 1.8. In all patients with a KT/V < 2.1 the dialysis regime is altered based on PD Adequest computer kinetic modelling to achieve a predicted KT/V > 2.09. To test the accuracy of the modelling we repeated the Adequest in 15 patients 4–8 weeks after the change in their dialysis regime. 5 patients had residual renal function, however this did not significantly decline between the 2 Adequests (p = 0.84). In all patients the KT/V improved, however the predicted KT/V differed from the actual KT/V by 0.12 (range –0.35 to 0.46). The predicted creatinine clearance differed from the actual by 3.4 L/week/1.73 m^2 (–9.0 to 19.34). There was no significant change in the serum albumin, another important marker of patient mortality (30.36 +/– 4.95 to 30.93 +/– 5.18, p = 0.77). There was no significant change in the serum creatinine, urea or body weight. In conclusion, the Adequest computer modelling can be used to improve KT/V and hopefully patient survival, however the Adequest should be repeated after any alteration in the dialysis regime.

P30

CD40-CD40L COSTIMULATION IS REQUISITE FOR BOTH THE INITIATION OF IMMUNE RESPONSES AND FOR EFFECTOR RESPONSES IN CRESCENTIC GLOMERULONEPHRITIS (GN)

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Planting an antigen in glomeruli of sensitised mice induces crescentic GN that resembles human disease. The requirement for CD40-CD40L in the generation of the nephritogenic immune response was determined by inducing GN in CD40–/– mice. Wild type (WT) mice developed crescentic GN with delayed type hypersensitivity (DTH) effectors in glomeruli. Conversely GN was attenuated in CD40–/– mice because of failure of lymphocyte priming. The role of CD40-CD40L in the effector phase was assessed in WT mice by an inhibitory anti-CD40L MoAb administered after the establishment of systemic immune responses. CD40-CD40L interaction was required in the effector phase of GN for glomerular DTH and crescent formation. CD40 was not constitutively expressed by kidney cells but was up regulated in glomeruli in the effector phase. Chimeric CD40–/– mice with intact bone marrow were constructed to assess the role of intrinsic renal cell expression of CD40 in the development of GN. Despite similar levels of skin DTH, glomeruli of CD40 chimeric mice did not demonstrate glomerular CD4+ T cell and macrophage infiltration compared to sham chimeras. This indicates that intrinsic renal cell CD40 may play a role in recruitment of DTH effectors to glomeruli in crescentic GN.

P32

OMAPATRILAT IMPROVES CARDIORENAL PATHOLOGY IN THE HYPERTENSIVE m(REN-2)27 RAT

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The present study investigated the cardiorenal effects of the vasopeptidase inhibitor omapatrilat, which simultaneously inhibits neutral endopeptidase (NEP) and angiotensin-converting enzyme (ACE), in the hypertensive transgenic (mRen-2)27 rat. Six-week-old male heterozygous Ren-2 rats were randomized to receive either no treatment, the ACE inhibitor fosinopril (10 mg/kg) or omapatrilat (10 or 40 mg/kg) by daily gavage. At 24 weeks, various functional and structural parameters were assessed. Results are shown as mean ± SEM except albuminuria shown as geometric mean. [a]p < 0.05 or [b]p < 0.01 versus control. [c]p < 0.05 or [d]p < 0.01 versus fosinopril. [e]p < 0.01 versus omapatrilat 10 mg/kg.

	Control (n = 9)	Fosinopril 10 mg/kg (n = 7)	Omapatrilat 10 mg/kg (n = 10)	Omapatrilat 40 mg/kg (n = 10)
Systolic Blood Pressure (mmHg)	178 ± 3	130 ± 4[b]	110 ± 3[bd]	91 ± 3[bde]
Renal ACE (% of control)	100 ± 3	81 ± 2[b]	66 ± 6[bd]	15 ± 2[bde]
Renal NEP (% of control)	100 ± 3	97 ± 3	84 ± 12[ac]	57 ± 2[bde]
Albuminuria (mg/24 hrs)	92.2	0.6[b]	0.3[b]	0.2[b]
Glomerulosclerotic Index (%)	1.7 ± 0.1	0.4 ± 0.1[b]	0.4 ± 0.1[b]	0.3 ± 0.1[b]
Medullary collagen staining (×100)	7.6 ± 1.0	2.2 ± 0.4[b]	2.1 ± 0.4[b]	0.8 ± 0.4[b]
Left Ventricular Weight (g/kg BW)	2.9 ± 0.1	2.2 ± 0.1[b]	2.0 ± 0.1[b]	1.8 ± 0.1[bd]

All treatments reduced systolic blood pressure and renal ACE. Omapatrilat induced a dose dependent inhibition of renal NEP, renal ACE and blood pressure. Renal injury as assessed by albuminuria, glomerulosclerotic index and medullary collagen staining was markedly attenuated by all treatments. Omapatrilat 40 mg/kg, was more effective than fosinopril in reducing left ventricular weight and blood pressure. The vasopeptidase inhibitor omapatrilat confers renal and cardiac protection in the hypertensive (mRen-2)27 rat. High dose omapatrilat may have superior organ protection compared to other antihypertensive agents partly via a blood pressure dependent mechanism.

P33

PULSE WAVE VELOCITY (PWV) EVALUATION DURING HEMODIALYSIS: EVIDENCE OF MEMBRANE BIOINCOMPATIBILITY

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Membrane bioincompatibility is one hypothesis for a high vascular mortality in hemodialysis patients. To directly evaluate vascular function aortic PWV, an independent ESRD mortality predictor, was measured before, during and after dialysis in 20 subjects using a polysulphone (Fresenius) and a polyamide S (Gambro) kidney. While PWV was unaltered with either, elastic vascular resistance (PWV^2/MAP) was increased during polysulphone dialysis ($p < 0.05$), an effect inversely related to time (months) on dialysis ($p < 0.001$). Analysis of 7 subjects with residual function and little change in BP during dialysis demonstrated an increase in PWV by 63% ($p < 0.01$) and 17% (NS) at 75 min with polysulphone and polyamide dialysis respectively (Fig. 1). These results demonstrate a potentially adverse direct effect of a commonly used kidney on aortic function and supports accumulating evidence that bioincompatible membranes contribute to vascular damage.

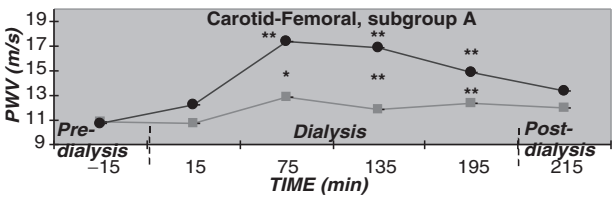

Fig. 1 The effect of dialysis on aortic PWV using polysulphone (●) and polyamide (■) kidneys in a patient subgroup ($n = 7$). *$p < 0.05$, **$p < 0.01$ between groups as well as compared to pre-dialysis measurements.

P34

THE IMPORTANCE OF GLUCOSE DEGRADATION PRODUCTS (GDP) IN FLUIDS FOR PERITONEAL DIALYSIS

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A patient on peritoneal dialysis (PD) uses 3–7 tons of PD fluids every year. This results in a considerable stress on the peritoneal tissue. Aspects of PD fluids that have been considered responsible for the bio-incompatibility are the low pH, the high osmolality, the high glucose and lactate concentrations and the presence of glucose degradation products (GDPs). The relative importance of each factor in PD fluids has however so far not been investigated. This was the aim of the present study.

Two main methods for investigating biocompatibility were used in this study: Cytotoxicity measured as *in vitro* inhibition of cell growth and *in vitro* AGE formation using albumin linked fluorescence.

The two most important factors for determining *in vitro* bio-incompatibility of PD fluids were the presence of GDPs that caused both severe cytotoxicity and strong AGE promotion and the low pH that induced severe cytotoxicity.

The biocompatibility of PD fluids may be monitored through fairly simple *in vitro* methods such as cell proliferation and AGE formation. Bio-incompatibility of PD fluids is mainly caused by the presence of GDP and the low pH. This correlates well with known clinical bio-incompatibility.

P35

EFFECT OF REUSE DIALYZER IN END STATE RENAL DISEASE PATIENTS WHO UNDERWENT HEMODIALYSIS IN Dr SARDJITO GENERAL HOSPITAL, YOGYAKARTA, INDONESIA

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Reuse dialyzer has been used commonly. In USA 81,5% of Dialysis Center have been used this way. This method is an effort to decrease cost of hemodialysis treatment. We has studied 48 End State Renal Disease (ESRD) patients who underwent twice a week regular hemodialysis in Dialysis Unit of Dr Sardjito General Hospital. All participants were divided into 2 groups. Group I is ESRD patients with non-reuse dialyzer (NRD) and group II is patients with reuse dialyzer (RD) for 5 times or more. We found no significant differences in: Osmolality value ($22\,500 \pm 18\,299$ *vs.* $31\,833 \pm 21\,959$; $p > 0.05$, 95%CI −4082 s/d −20 748), ureum value ($75\,611 \pm 24\,577$ *vs.* $82\,333 \pm 26\,133$; $p > 0.005$, 95%CI −8626 s/d −22 070), and creatinine value (7505 ± 3036 *vs.* 7053 ± 1876; $p > 0.05$, 95%CI −1876 s/d −0.972). From clinical we found itching on 16 patients (RD) vs. 4 (NRD); $p > 0.05$, 95%CI 0.053–1.094. Nausea 8 (RD) vs. 5 (NRD); $p > 0.05$, 95% CI 0.235–4688. Headache 7 (RD) vs. 10 (NRD); $p > 0.05$, 95% CI 0.993–17 791. We concluded that reuse dialyzer 5 times or more give same laboratory findings as non-reuse dialyzer. Reuse dialyzer decreased first use syndrome.

P36

USE OF ACUTE VASCULAR ACCESS CATHETERS (AVACs): A SURVEY OF THE IMPLICATIONS

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The use of AVACs has facilitated delivery of haemodialysis to patients lacking functioning established access. We retrospectively reviewed our experience of more than 156 separate AVACs between July 2000 and May 2001, totalling 3666 patient days. AVACs were inserted as access for ARF (11%), CRF (23%), failed arteriovenous grafts and fistulae [AVF] (21%), failed CAPD (10%) or failed prior AVAC (35%). The majority of AVACs were placed on the right side (78%) and the placement site was [in order of unit preference] tunnelled (18%), jugular (67%), subclavian (3%) or femoral (12%) in acute or urgent situations.

During the period of follow up 139/156 of the AVACs inserted were subsequently removed, with the rest still in-situ at the end of follow-up. The mean survival days were 7.7 + 6.6 days for femoral AVACs, 11.6 + 8.1 days for subclavian, 21.5 + 24.2 days for jugular and 44.8 + 31.8 days for tunnelled AVACs. Tunnelled AVACs provided survivals that were statistically significantly longer than other AVAC sites. Causes for AVAC removal were: elective (56%), obstruction (27%), infection (14%) and a few with cracked catheter (2%). Elective causes included return to AVF use or CAPD or replacement of femoral by jugular AVAC.

The issue of infection was monitored by routine post-removal tip culture. Tip cultures grew out MRSA (11%), CNS (44%), other Staph (9%) or gram -ve rods (1%) although the majority of tips showed no positive culture (35%). Blood cultures were performed in 33/156 patients, for usual clinical indications. Blood cultures were negative in a significant proportion (46%) but the rest grew out MRSA (21%), CNS (6%), other Staph (24%)or very rarely E coli (3%). Patients with positive blood culture or significant tip culture result were deemed to have been infected (32/156), however infection was only quoted as a clinical cause for removal in 14% (as above)and Kaplan-Meier estimates of catheter survival did not show significant differences in AVAC survival between infected and non-infected.

Reviewing AVAC use and sequelae facilitates the process of quality improvement by providing benchmarking data and identifying areas requiring additional focus and resources. AVAC survival is reduced by the complications of obstruction and infection in almost 50% of patients and manouevres to address these problems may improve the usefulness of AVACs.

P37

BIOCOMPATIBILITY ASPECTS OF THE GRADIFLOW IN RENAL DIALYSIS

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Gradiflow has previously been shown to have potential in renal dialysis and has demonstrated the effective removal of nitrogenous waste metabolites and phosphate ions from human plasma and whole blood samples. The Gradiflow is a membrane-based preparative electrophoresis system in which separations are achieved using the dual strategy of charge and size.

The distinguishing features of this technology are a set of polyacrylamide membranes and the application of an electrical potential across these membranes. The use of these features allows the selective removal of contaminants from biological sources. This beckons the question of the biocompatibility of the membranes and the effect that voltage has on cellular and biochemical components of human plasma and blood.

To answer this question, tests on hemolysis, coagulation, complement and cellular activation were conducted. Results show that the Gradiflow does not have significant effects on hemolysis, coagulation and complement activation. Flow cytometry results indicate that platelets and white cells were not adversely affected.

These results indicate that the Gradiflow has significant potential in the treatment of renally compromised patients and may have therapeutic uses in other treatment modalities requiring dialysis.

P38

BEHAVIOUR OF BETA-2-MICROGLOBULIN IN THE GRADIFLOW

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Beta-2-microglobulin (β2m) associated amyloidosis is a serious and debilitating complication that affects long term dialysis patients. Current dialysis treatments are unable to remove β2m effectively, which leads to its accumulation and deposition as part of amyloid fibrils in various musculoskeletal structures. Similarly, hyperphosphatemia is an important biochemical consequence of renal failure, which is only partially improved by current dialytic therapies.

The Gradiflow is a novel separation technology that is able to rapidly separate macromolecules such as proteins, nucleotides and complex sugars based on size and/or charge. The efficacy of this technology in removing β2m and phosphate was investigated in chronic haemodialysis patients. To do this, β2m of individual patients was characterised, and the removal kinetics of β2m and phosphate for each patient determined.

Preliminary results indicate that the Gradiflow is capable of removing both beta-2-microglobulin and phosphate. There was a 55% decrease in patient plasma β2m levels and a 98% reduction of phosphate levels in spiked plasma samples over an hour. The rate of removal of β2m was dependent upon the pore size of the separation membrane used and the applied voltage.

The separation capability of the Gradiflow in removing commercially available human β2m dissolved in various buffers was also examined. However, it was ascertained that the commercially available β2m was not representative of the native state of β2m found in haemodialysis patients.

These results suggest that the Gradiflow may offer a novel method to assist current renal dialysis systems in the reduction of β2m and phosphate, and as such may be a powerful new therapeutic alternative for the prevention and treatment of complications of renal failure.

P39

THE EFFECT OF OBESITY ON RENAL TRANSPLANT OUTCOMES

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Although obesity has been shown to be associated with improved survival on dialysis, its effects on renal transplant outcomes remain unclear. The present study aimed to evaluate the effect of obesity on both short- and long-term renal transplant outcomes. A retrospective analysis was undertaken of all adult patients transplanted at our centre (1994–2000). Patients were rigorously screened for cardiovascular disease prior to acceptance for transplantation. The effects of obesity on renal transplant outcomes were assessed by binary logistic and multivariate Cox regressions. Of the 493 patients transplanted, 59 (12%) were obese (BMI > 30 kg/m^2). Obese patients were more likely to experience wound breakdown (4% vs 17%, $p < 0.01$) and wound infections (8% vs 15%, $p = 0.10$). There were no significant differences between non-obese and obese recipients with respect to operating times, wound drainage, delayed graft function, medical complications, urologic complications, hospital stays or readmissions, and acute rejection episodes. Five-year actuarial survival rates were comparable between the two groups with respect to graft survival (83% vs 84%, p = NS) and patient survival (91% vs 91%, p = NS). BMI was an independent risk factor for wound breakdown ($p < 0.001$), but not for other post-transplant complications, hospitalisation, graft loss or patient survival. In conclusion, the only significant adverse effect of obesity on renal transplant outcomes was an increase in wound complications, which were generally of minor consequence. Provided that adequate care is taken to avoid transplanting patients with significant cardiovascular disease, obese recipients can achieve excellent long-term patient and graft survivals that are comparable with their non-obese counterparts. Denying patients access to renal transplantation on the basis of obesity *per se* is not justified.

P40

ICODEXTRIN AS SALVAGE THERAPY IN PERITONEAL DIALYSIS PATIENTS WITH REFRACTORY FLUID OVERLOAD

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Icodextrin is a high molecular weight, starch-derived glucose polymer, which is capable of inducing sustained ultrafiltration over prolonged (12–16 hour) peritoneal dialysis (PD) dwells. In this study, the ability of icodextrin to alleviate fluid overload was prospectively evaluated in 17 PD patients (8 females/9 males, 12 CAPD/5 APD, mean age 56.8 ± 2.9 years) who were on the verge of being transferred to haemodialysis because of symptomatic fluid retention that was refractory to fluid restriction, loop diuretic therapy, hypertonic glucose exchanges and dwell time optimisation. One icodextrin exchange was substituted for either 1 overnight (CAPD patients) or 1 daytime (APD patients) glucose exchange. The group contained a large proportion of diabetics (7 type I and 5 type II) and patients with high and high average membrane transport characteristics (88%). Icodextrin significantly increased daily peritoneal ultrafiltration (885 ± 210 ml to 1454 ± 215 ml, $p < 0.05$) and reduced mean arterial pressure (106 ± 4 to 96 ± 4.2 mmHg, $p < 0.05$), but did not affect weight, plasma albumin concentration or dialysate : plasma creatinine ratio. Diabetic patients also experienced improved glycaemic control (haemoglobin A1c decreased from 8.9 ± 0.7% to 7.9 ± 0.7%, $p < 0.05$). Overall PD technique survival was prolonged by a mean of 11.6 months (95% CI 6.0–17.3 months). On multivariate Cox proportional hazards analysis, extension of technique survival by icodextrin was significantly predicted by diabetic status (hazard ratio [HR] 0.11, 95% CI 0.01–0.99, $p < 0.05$) and baseline net peritoneal ultrafiltration (HR 3.77, 95% CI 1.24–11.5, $p < 0.05$). In conclusion, icodextrin can significantly improve peritoneal ultrafiltration and extend technique survival in PD patients with symptomatic fluid overload, especially those who are diabetic or have substantially impaired peritoneal ultrafiltration.

P41

PREVALENCE, RISK FACTORS, AND FRACTURE RISK FOR REDUCED BONE DENSITY IN DIALYSIS PATIENTS

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BACKGROUND

Dialysis patients are at risk for reduced bone mineral density (BMD) due to renal osteodystrophy and osteoporosis. The risk may be exacerbated by advancing age, hypogonadal status, sedentary lifestyle, and hypogonadal status, sedentary lifestyle, and hyperparathyroidism. Dialysis patients have also been shown to have a 3–4 fold higher risk of hip fracture than the general population. Dual energy x-ray absorptiometry (DEXA) is a reproducible measure of BMD and is used by the WHO to define osteopenia ($-2.5 < T \leq -1.0$) and osteoporosis ($T \leq -2.5$). This study assessed the prevalence and risk factors for reduced BMD in the dialysis population as well as the prevalence of fracture.

METHOD

Subjects underwent DEXA measurement of BMD at the lumbar spine and femoral neck. Potential risk factors for reduced BMD were recorded. Two tailed Pearson correlation analysis was performed as well as linear regression analysis to determine risk factors for reduced BMD.

RESULTS

114 (38% female) chronic dialysis patients (82.8% haemodialysis) of mean age 60.7 +/– 16.8 years were studies. 43% had osteoporosis, 40% osteopenia, and 17% normal BMD at the hip. Significant positive correlations were found with height, weight, urine volume, Karnofsky score, heparin dose, and use of calcium based phosphate binders. Significant negative correlations were found with aged, female sex, parathyroid hormone level, and urea reduction ratio. 60% of peritoneal dialysis patients, and 39% of haemodialysis patients had osteoporosis at the hip ($P = 0.08$). The rate of fracture in the osteoporosis group was 22% compared with 9% in the other groups ($P = 0.05$). Stepwise regression multivariate analysis found aged and weight predict 71% of the variation in hip BMD in this cohort.

CONCLUSION

Reduced BMD is very common in the chronic dialysis population. It is associated with many variables, particularly advancing age and low weight. BMD in the osteoporosis range at the hip is associated with an increased fracture rate.

P42

A COMPARATIVE STUDY OF UREA-N CLEARANCE AND UREA-N REDUCTION RATIO BETWEEN NEW DIALYZER AND RE-USED DIALYZER

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BACKGROUND: In Indonesia, hemodialysis is now costly. It was shown that a re-used dialyzer had some benefits such as cost-efficient and better biocompatibility. However, this dialyzer has decreased effectiveness as well as a higher rate of contamination by bacteria and disinfectant.

STUDY OBJECTIVES: 1. To compare urea-N clearance and urea reduction ratio (URR) between new (first use) dialyzer and re-used dialyzer. 2. To know the maximum use of dialyzer effectively.

PATIENTS & METHOD: A comparative study was conducted in the HD Unit of Dr. Kariadi Hospital between September 1999 to January 2000. Subjects were patients with chronic renal failure undergoing hemodialysis.

RESULTS: Twenty-one patients were included in the study, consisted of 16 males (76%) with range of age 20–59 years. Mean (±SD) urea-N clearance of new dialyzer was 126 (±38.9) ml/minute. The average compartment content was 70 ml in the new dialyzer and 67.7 ml in the third used dialyzer, a decrease of 3.2 ml. The re-used dialyzer did not decrease urea-N clearance at the final use (multiple $R = -0.161$; $p = 0.48$) and at the average use (multiple $R = -0.024$; $p = 0.91$). The re-used dialyzer did not decrease URR at the final use (multiple $R = -0.247$; $p = 0.28$) and at the average use (multiple $R = -0.087$; $p = 0.69$). The decrease of urea-N clearance has started at the fourth use (B unstandardized coeff. –0.087) and decrease of urea-N clearance has started also at the fourth use(B unstandardized coeff. –0.184). CONCLUSION: The re-used dialyzer did not decrease urea-N clearance and URR. The application of the re-used dialyzer might be stop at the fourth use since at this time-point the decrease of urea-N clearance and URR was started.

P43

ASSESSMENT OF PLATELET FUNCTION IN HAEMODIALYSIS PATIENTS

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Renal failure is typically considered as being associated with a uremic platelet defect wherein platelet numbers are normal but platelet function is impaired. On the other hand platelet thrombus formation within dialysers is a common clinical problem and platelets are associated with the intimal hyperplasia seen in synthetic graft fistulae. We wished therefore to assess platelet function in a group of haemodialysis (HD) patients. In the first part of the study, 8 stable HD patients were assessed pre and post a mid-week dialysis session. All dialysis sessions were 4 hours on Fresenius machinery using low- or high-flux polysulfone membranes. The following were assessed: Factor VIII concentration (FVIIIc), Von Willebrand antigen (VWAg), Prothrombin fragments 1&2 (PF1&2—indicative of in-vivo thrombin generation), D-dimer (DD—indicative of fibrinolysis), P-selectin (PAMS or CD62) and CD63, as well as total plasma homocysteine (Hcy). The platelet count was normal in all patients. There were mixed results for FVIIIc and VWAg with some patients having normal and some elevated levels; likewise dialysis had no consistent effect on levels. D-dimer and CD63 levels were similarly inconsistent. PF1&2 was elevated in all but 1 patient but the dialysis procedure did not influence the levels. The patient with normal levels was the only patient who had a normal level of plasma Hcy. PAMS was detectable in the normal range for all patients both pre and post-dialysis but demonstrated a typical concentration dependent increase with increasing doses of ADP, suggesting that the platelets were able to be activated in a normal fashion. Dialysis improved the response to ADP with higher levels of expression of CD62. To further explore the possible association of Hcy levels with PF1&2, a total of 37 HD patients were examined (and 10 CAPD patients were also examined) but no correlation of PF1&2 and Hcy levels was found (including after log transformation of the data). The CAPD patients also exhibited elevated PF1&2 levels.

These results demonstrate that baseline platelet function is abnormal in dialysis patients in an inconsistent pattern but that the response to stimuli such as ADP is normal and is further augmented by dialysis. In addition, thrombin generation is increased and this may contribute to vascular thrombosis in these patients.

P44

PHARMACOKINETICS OF FRAGMIN, CLEXANE AND ORGARAN IN STABLE CHRONIC HAEMODIALYSIS (HD) PATIENTS

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An infusion of unfractionated heparin (UH) is commonly used for anticoagulation during HD. Low molecular weight heparin (LMWH) can instead be given as a bolus before dialysis. Orgaran (Danaparoid sodium) is also an alternative to UH but is used mainly for patients with type II heparin-induced thrombocytopenia. There is little data on the duration of action or whether accumulation occurs with time in these renally excreted drugs. We thus performed a prospective randomised study of Clexane & Fragmin at our routinely prescribed lower doses plus Orgaran at its recommended dose in stable HD patients. After a 2 week (W) run-in period using UH, 21 patients were randomised to Clexane 40 mg (mean dose 0.7 mg/kg), Fragmin 2500 u (mean dose 39 u/kg), or Orgaran 34 u/kg for a period of 4 W. Anti-Factor Xa (AFXa) levels were measured at baseline and at the end of W1 and W4 at the following times: prior to injection & 1, 2, 3, 4, 24, & 48 hours post injection. Urea reduction rate (URR), FBE and coagulation profiles were measured at baseline and at W1 & 4. At the end of each session the dialyser and puncture holes were visually inspected for evidence of clotting and bleeding respectively. 17 patients completed the study. Anti-factor Xa levels (mean ± SEM) for Fragmin, Clexane & Orgaran 4 hr post injection at W1 were 0.20 ± 0.04, 0.38 ± 0.03 & 0.54 ± 0.05 units ($p = 0.0002$, Orgaran vs others) and at W4 were 0.26 ± 0.04, 0.40 ± 0.06 & 0.64 ± 0.05 ($p = 0.0005$, Orgaran vs others). Orgaran levels during dialysis were also higher at W4 compared to W1 (1 hr: $p = 0.03$, 2 hr: $p = 0.003$, 3 & 4 hr: $p = 0.01$) whereas levels for Clexane and Fragmin were unchanged. AFXa levels for Orgaran were higher than for Clexane and Fragmin from 2 hr to 48 hr post injection at W1 ($p < 0.0001$ to $p < 0.03$) and from 3 hr to 24 hr at W4 ($p < 0.0001$ to $p = 0.004$). There were no episodes of line or dialyser clotting or bleeding episodes during the study. There were no differences in URR or haemoglobin levels between groups and no adverse events were seen. Fragmin and Clexane provided appropriate anticoagulation at lower than recommended doses, with minimal duration of action beyond 4 hrs and no evidence of drug accumulation. Orgaran, at the current recommended dosage, resulted in significantly higher anticoagulation levels and drug accumulation than Clexane and Fragmin, suggesting both dose reduction and monitoring of AFXa levels with treatment are appropriate.

P45

INSERTION OF PERITONEAL DIALYSIS CATHETERS USING Y-TECH SCOPE AND PROCEDURE—2 YEAR EXPERIENCE AT TAMWORTH BASE HOSPITAL

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Background: Tenchkoff catheters for peritoneal dialysis are usually inserted via laparotomy or laparoscopy. These procedures usually require a general anaesthetic and a varying length of stay from 2–7 days. Insertion of catheters with a Y-Tech scope is performed under local anaesthetic as a day only procedure. We report our experience over a 2 year period using Y-Tech insertion. Of a total of 25 Swan Neck catheters inserted 23 were inserted using Y-Tech. Of the 2 patients using laparotomy one had known adhesions and the second an umbilical hernia needing repair. On 2 occasions early in the learning curve the catheters were inserted extra peritoneal and required repositioning of the peritoneal segment via laparotomy. All the other catheters have worked without flow problems. Of the 23 patients 21 remain on PD two patients changing to HD after peritonitis unrelated to catheter insertion [91% catheter survival]. There has been one early exit infection and in this patient the external cuff has eroded. Of the 16 catheters inserted electively 14 patients have had day only admissions two patients [numbers 1 and 3] stayed overnight. There have been no surgical complications and no late peri-catheter herniae. In conclusion the Y-Tech method of catheter insertion is advantageous in that it is convenient allowing catheter insertion at time of need rather than tagged onto surgical lists, cost effective and safe both in this small and other larger published series.

P46

A TOTAL BODY NITROGEN PREDICTION EQUATION FOR DIALYSIS DEPENDENT PATIENTS

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Malnutrition is a common problem in patients with end-stage renal failure and is associated with significant morbidity and mortality. Nutritional state can be difficult to assess accurately in patients with renal disease. Total body nitrogen (TBN) is considered to be the gold standard of nutritional assessment as it is directly proportional to body protein. However, TBN is not readily available for routine clinical use. The aim of the present study was to assess the validity of using simple anthropometric measurements in determining body protein. Patients from Royal North Shore Hospital Sydney, who had undergone simultaneous total body nitrogen assessment along with a detailed anthropometric evaluation, were studied. Using the data on 283 patients a TBN prediction equation was derived using age, height, weight, sex (M=1, F=0), sum of the skinfold thickness and a sex and weight interaction term as follows: TBNp=−722.47−3.98*Age+9.27*Ht+14.23*Wt−401.53*Sex+9.42*SexWt−2.34*SUMSF (R^2=0.89). This equation was then evaluated against the observed TBN results of the next 109 consecutive patients using a method described by Bland and Altman. A significant difference was found (78 grams, P=0.002) with wide limits of agreement (−395 to 552 grams). Thus although the prediction equation could determine mean TBN within 78 grams of the actual mean TBN the large limits of agreement are likely to diminish the clinical utility of predicting body protein from anthropometric measurements in patients on dialysis.

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A LONGITUDINAL COMPARISON OF NUTRITIONAL STATE IN PATIENTS TREATED WITH PERITONEAL DIALYSIS COMPARED TO HAEMODIALYSIS

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Controversy exists as to which modality of dialysis should be chosen when dialysis is first commenced. It is known that nutritional state can have a significant effect on subsequent morbidity and mortality. Thus the effect of dialysis modality on nutritional state should be considered. Both peritoneal dialysis (PD) and haemodialysis (HD) can theoretically effect nutritional status through treatment related protein and amino acid losses and bio-incompatibility of either the dialysate or dialysis membrane. We aimed to further explore the treatment effect of dialysis modality on patients subsequent nutritional state.

From 1990 patients on PD and HD in our department have undergone regular assessment of nutritional status using direct measurement of protein stores by in vivo neutron capture analysis (TBN). In this analysis we selected only those patients who had >=3 assessments of TBN over a period of greater than 1 year on a given dialysis modality. The rates of change in TBN (grams) for each patient was determine from the slope of the plot of TBN by dialysis duration (months). The difference in these slopes between patients on PD and HD were then compared using an unpaired t-test. One hundred and forty four patients met the analysis criteria (PD=51, HD=93). Both dialysis modalities were associated with a positive mean increase in TBN over time (PD=4.9 grams/month, HD=1.8 grams/month) however, this was not statistically significant (P=0.1). There was no significant difference in TBN result between the two groups at their first assessment (mean difference=18.8 grams, P=0.8).

Thus although patients on PD increased body protein at a greater rate than that observed in the HD population, no firm recommendation can be made regarding a nutritional benefit of one dialysis modality over the other.

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THE EFICACY OF DIALYZER REUSE IN CHRONIC HEMODIALYSIS ESRD PATIENTS

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Clinical trial was done to investigate the eficacy of dialyzer reuse in Hemodyalisis endstage renal disease patients in Nephrology ward Mohamad Hoesin Hospital Palembang, Indonesia. All patients run hemodialysis five hours twice a week using acetate dialysat and Fb-110 TGA dialyzer. The frequency of dialyzer reuse was depend on the value of priming volume (more than 80%). The parameter of eficacy were Kt/v (>1.8) and URR(>65%). We done manual proceedure of dialyzer reuse.

Twenty patients (10 were male) included this study. The range of age was 41–50 years and the average of lenght of hemodyalis was 3.45 years. The average of frequency of dialyzer reuse was 5.9 times (3–11 times). There were no association between Kt/v and the frequency of dialyzer reuse. Of 114 hemodialysis event using dialyzer reuse with priming volume >80%, 83.3% achieved URR > 65%.

In conclusion, 83.3% of hemodialysis patients using dialyzer reuse achieved URR more than 65%.

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A COMPARISON OF PERITONEAL CATHETER-RELATED INFECTION RATES BETWEEN ABORIGINAL AND NON-ABORIGINAL PATIENTS IN PREVALENT CAPD PATIENTS

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Outcome and determinants of catheter-related infection (Exit Site Infection (ESI) and Peritonitis (P) were determined in 73 current CAPD patients [34 Aboriginal and 39 Caucasian or Asian] with a median duration of CAPD of 478 days who had baseline metabolic, dialysis adequacy and demographic data captured at 4 weeks. The Aboriginal patients were younger with a greater prevalence of diabetes than Non-Aboriginal patients (mean aged 50 vx 60 and diabetes 59% vs 36%) and were largely resident in remote communities. Increased CRP (>5 mg/L) at baseline was found in a higher proportion of Aboriginal patients (76%) vs non-Aboriginal patients (44%). The mean haemoglobin and albumin at entry in the Aboriginal group were 109 g/L and 34 g/L respectively compared with 113 g/L and 37 g/L in the Non-Aboriginal group. There was a significantly increased composite catheter-related infection end-point rate (ESI or P) in the Aboriginal patients vs Non-Aboriginal (224 days vs 506 days $P<0.05$). There was no difference in catheter-related infection rates between diabetic and non-diabetic groups. In the combined patient group, patients with an entry (4 week) CRP value of <5 had 0.77 event per patient treatment year compared with 1.7 events per patient in those patients with CRP value of >5 ($p<0.05$). Additional factors that might influence catheter infection event rates, including race, diabetes, aged, BMI, albumin, CRP, were entered into a Cox Proportional hazards model in order to identify predictors of catheter infection and will be presented. Aboriginal patients maintained on CAPD in Western Australia have significantly greater infection-related events than Non-Aboriginal Patients, which may be related to adverse environmental and inflammatory changes.

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SMOKING IS ASSOCIATED WITH MICROALBUMINURIA AND DECREASED CREATININE CLEARANCE IN A NON-DIABETIC POPULATION—THE RENAL AUSDIAB STUDY

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Population studies have described an association between smoking and both albuminuria and decreased renal function but these have not clearly excluded subjects with diabetes and impaired glucose tolerance (IGT). We report such an association in a population survey where a glucose tolerance test was used to exclude subjects with both IGT and diabetes mellitus. Ex smokers, diabetics and subjects with IGT were excluded. The AUSDIAB study collected a spot urine sample from participants for calculation of the urinary albumin to creatinine (alb : creat) ratio. A total of 5302 subjects were included in the analysis (current smokers n=1187 and never smokers n=4115). Creatinine clearance (CrCl) in mL/min was calculated using the Cockroft and Gault formula, with 0.85 adjustment for women subjects. Subjects with urinary alb : creat ratio ≥3.4 (n=251) were compared to those with urinary alb : creat ratio <3.4 (n=5051), and subjects with CrCl less than 60 mL/min (n=531) were compared to those with CrCl above 60 mL/min (n=4771). Backwards step-wise logistic regression analysis with co-variates including age, sex, blood pressure, serum lipids, fasting blood glucose, BMI, waist circumference and smoking status was used. Odds ratio for current smokers compared to never smokers for urinary alb : creat >3.4 was 1.76 (95% CI: 1.29, 2.39) and for CrCl <60 mL/min was 1.68 (95% CI: 1.15, 2.46), independent of all other co-variates included in the analysis. This cross-sectional study shows an independent association between current smoking status and microalbuminuria and reduced creatinine clearance.

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ORAL FLUCLOXACILLIN FOR STAPHYLOCOCCUS AUREUS INFECTION IN DIALYSIS DEPENDENT PATIENTS

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Effective treatment of S. aureus infection requires 2 weeks of intravenous (IV) antibiotic therapy. The increasing incidence of vancomycin resistance has lead to the recommendation that glycopeptides use be limited. Isoxazolyl penicillins are an alternate option for treatment of S. aureus infections. However use of IV isoxazolyl penicillins would mean prolonged IV access and inpatient stays for patients. Flucloxacillin and dicloxacillin are renal excreted. In patients with renal failure, oral flucloxacillin may achieve concentrations which are bactericidal for S. aureus infection.

Three groups of 7 subjects were recruited. Group 1 were patients with normal renal function, S. aureus infection, on IV flucloxacillin, 2 gms q6 hours. Group 2 were dialysis dependent volunteers, no active infection, who received oral flucloxacillin 500 mg q8 hours. Group 3 were dialysis dependent volunteers, no active infection, who received oral flucloxacillin 1 gm q8 hours. After 3 days of treatment, 10 serum samples were obtained from each subject, over a 6 hour (Group 1) or 8 hour (Groups 2 and 3) dosing interval. Free flucloxacillin concentrations were determined.

Flucloxacillin concentrations for each patient were used to determine the area under the curve (AUC) and the time spent above the MIC 90 for S. aureus (0.5 mg/L). These were then estimated for a 24 hour period and compared.

The AUC for each group was statistically similar (1040 +/– 680 mg/L/min, Group 1; 685 +/– 285 mg/L/min, Group 2; 894 +/– 381 mg/L/min, Group 3). The time above the MIC 90 of S. aureus was statistically similar (634 +/– 290 min, Group1; 622 +/– 340 min, Group 2; 921 +/– 427 min, Group3).

We conclude that oral flucloxacillin, in patients renal failure, achieves concentrations are bactericidal and comparable to those routinely used to treat S. aureus infections.

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GENETIC FACTORS MEDIATING PROTECTION AGAINST OXIDATIVE STRESS AND NON-MELANOMA SKIN CANCER ACCRUAL RATES IN RENAL TRANSPLANT RECIPIENTS

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Non-melanoma skin cancer (NMSC) represents a signficant cause of morbidity and mortality among renal trasnplant recipeints (RTR). Not all RTR develop NMSC and in those who do, the rate of accrual varies considerably. It is unlikely that UV exposure alone explains this variant. Polymorphism in member sof the glutathione S-transferase (GST) supergene family is associated with altered NMSC risk in non-transplant patients. We examined allelism in GSTM1, GSTP1, GSTM3 and GSTT1 in 398 RTR at PAH (mean age at transplantation 41.0±14.2 years, mean follow up 8.6±7.4 years). Structured interview and full skin examination were performed; 198 developed 4080 histologically-proven NMSC. 1834 invasive squamous cell basal cell carcinoma (BCC) in 151, 234 keratacanthoma in 65, and 1 sweat gland tumour. NMSC was associated with fair skin, red/blond hair, hazel/blue eyes, duration of immunosuppression, male gender and outdoor occupation. Negative binonial regression analysis showed that, compared with GSTM1 AB, GSTM1 null was associated with increased numbers of both SCC (RR 7.14, 95% CI 1.67–33.33; p=0.008) and BCC (RR ••

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A RANDOMISED CONTROLLED TRIAL OF TOPICAL EXIT SITE MUPIROCIN APPLICATION IN PATIENTS WITH CUFFED HAEMODIALYSIS CATHETERS

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Central venous catheters are frequently needed for the provision of haemodialysis, but their clinical usefulness is generally limited by infectious complications. The risk of such infections can be reduced by topical application of mupirocin to the exit sites of non-cuffed catheters or by the use of cuffed catheters. Whether mupirocin offers any additional protection against infection in patients with cuffed haemodialysis catheters has not been studied. To address this issue, an open-label, randomised controlled trial was performed comparing the effect of thrice-weekly exit site application of mupirocin versus no ointment on infection rates and catheter survival in incident patients receiving haemodialysis via a cuffed central venous catheter. The trial was terminated prematurely following an interim analysis of results obtained from the first 50 enrolled patients. Both the mupirocin (n = 27) and control (n = 23) groups were similar at baseline with respect to demographic characteristics, comorbid illnesses and causes of renal failure. Compared with controls, mupirocin-treated patients experienced significantly fewer catheter-related bacteraemias (7.4% versus 39.1%, $p < 0.01$) and a longer time to first bacteraemia (log rank score 8.68, $p < 0.01$). The beneficial effect of mupirocin was entirely attributable to a reduction in staphylococcal bacteraemia (log rank 10.69, $p = 0.001$). Median [interquartile range] catheter survival was significantly longer in the mupirocin group (40 [27–64] days versus 24 [13–33] days, $p < 0.05$). Mupirocin use was not associated with any adverse patient effects or the induction of antimicrobial resistance. In conclusion, thrice-weekly application of mupirocin to cuffed haemodialysis catheter exit sites is associated with a marked reduction in line-related sepsis and a prolongation of catheter survival.

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PREVENTION OF TUNNELED HAEMODIALYSIS CATHETER (TC) RELATED INFECTIONS USING CATHETER RESTRICTED FILLING WITH GENTAMICIN AND CITRATE

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Background: TC?s are an essential form of short- and long-term haemodialysis (HD) access. However, TC-related infections (TCI) are associated with sepsis, loss of access and prolonged hospital admissions.

Objective: To determine the efficacy of catheter-restricted filling with gentamicin and citrate compared to standard heparin in preventing TCI?s.

Design: 2 year multicentre, double-blind randomised trial (1999–2001)

Method: Patients on or starting HD having a TC inserted were randomised to receive heparin or gentamicin/citrate. Exclusions: pre-existing sepsis or antibiotics; gentamicin/citrate allergy. TC function was monitored by assessing blood flow. TCI?s were the primary end-point.

Results: 100 TC?s in 71 patients were randomised. Reasons for line insertion were: 40% start dialysis (no other access), 17% failed fistula, 6% failed CAPD, 37% re-insertion (73% due to inadequate blood flow). 71% received pre-insertion prophylactic antibiotics. Median TC use was 31 days (1 ? 438 days) and a total 5170 catheter days have been accrued so far. There were 10 episodes of asymptomatic colonization of TC, 6 exit-site infections, 3 definite blood-stream infections (BSI) and 4 probable BSI. Rate per 100 catheter days translates to 0.44 all TCI?s, 0.25 symptomatic TCI?s and 0.14 TC-related BSI. Follow-up concludes on June 30 2001 after which the randomisation schedule will be opened.

Conclusion: overall, our tc-related bsi rate compares favourably with published reports. To our knowledge this study represents the first controlled observation in australia and world-wide. Results on the efficacy of gentamicin/citrate will be available in july 2001.

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INCREASING HbA1c WITHIN THE NORMAL RANGE IS ASSOCIATED WITH ALBUMINURIA IN NON-DIABETIC ABORIGINAL AUSTRALIANS

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Albuminuria is common in NT Aboriginal communities, where it marks risk of cardiovascular as well as renal disease. Type 2 diabetes is also common. Here we report the association between albuminuria and indicators of glycaemia among people without diabetes in a community with high rates of ESRD.

We performed a population based survey (n = 237, 55% adult population) assessing renal disease, carotid intima-media thickness (IMT) and atherosclerotic risk markers (including random glucose, HbA1c and spot urine albumin/creatinine ratio -ACR). ACR was categorised into normal (< 3.4 g/mol), microalbuminuria (MA-ACR 3.4–33.9) and overt albuminuria (OA-ACR 3.4–34). Excluded were those with a history of diabetes or a random glucose > 11.1 or fasting glucose > 8.0 mmol/l.

Of the 168 remaining, mean HbA1c was 5.61 ± 0.58%, with a range from 4.3 to 7.4%. Albuminuria was prevalent in this non-diabetic (ND) group (25% MA, 8% OA). ACR was related monotonically to HbA1c, increasing by a factor of 2.0 [1.3–3.1] per 1% rise in HbA1c ($r = 0.25$, $p = 0.001$).This trend remained when analysis was restricted to HbA1c < 6.5%. ACR was also significantly associated with age, female sex, BP, C-reactive protein (CRP), BMI, waist hip ratio (WHR) and increasing IMT. HbA1c was significantly associated with waist hip ratio ($p = 0.001$), age ($p < 0.001$), diastolic BP ($p = 0.02$), but not BMI, CRP, fibrinogen or homocysteine. HbA1c was associated with increasing IMT, but not after adjustment for age. In a multivariate analysis the relationship between HbA1c and ACR in ND was independent of age, WHR, and BP (ACR rose by a factor of 1.3 [1.2–1.5] per 1% change inHbA1c). Calculated GFR was inversely related to HbA1c, falling 9.0 [3.5–14.5] ml/min/1.73 m^2 per 1% HbA1c ($r = -0.25$, $p = 0.001$). This was not however independent of age.

In this environment increases in ACR are associated with HbA1c within the 'normal' range, as well as BP, obesity and increased cardiovascular risk. It suggests the mechanisms underlying hyperglycaemia and albuminuria and thereby the excess of renal and cardiovascular disease operate over the high-normal degrees of glycaemia.

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BLOCKADE OF P38 ALPHA MAPK SIGNALING AMELIORATES RENAL INJURY IN ACUTE RAT ANTI-GBM GLOMERULONEPHRITIS

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P38 mitogen activated protein kinase (MAPK) is a critical intracelluar signal transduction pathway involved in inflammation. Activation of the p38 pathway induces a variety of inflammatory mediators including production of cytokines and chemokines. Little is known regarding p38 MAPK signalling in kidney disease. Therefore, we investigated the potential role of this pathway in rate accelerated anti-GBM disease. Accelereated anti-GBM disease was induced in groups of 5 SD rats, with animals killed at 3 hours and 1 day after administration of nephrotoxic serum. Western blotting of glomerular lysates demonstrated an increase in phosphoryiated (active) p38 at 3 hours and day 1. Double immunostaining identified glomerular expression of phosopo-p38 in podocytes, lymphocytes, neutrophits and macrophages. To further elucidate the functional role of p38, groups of 5 animals were given a novel inhibitor of the p38 alpha isoform (NPC 31 145 40 mg/kg bid), or vehicle control, 2 hours prior to administration of nephrotoxic serum. Animals were killed at 3 hours or day 1. p38 blockade significantly reduced proteinuria (114 ± 48 vx 258 ± 72 mg/24 hr in control animals; P,0.001), and prevented a rise in serum creatinine (49 ± 8 vs 77 ± 11 umol/L in controls; $P < 0.001$, normal 42 ± 11 umol/L). Next, we analyzed glomerular leukocytic infiltation at 3 hours as renal injruy is leukocyte dependent in this model. p38 blockade significantly reduced glomerular neutrophil accumulation (7.5 ± 3.13 vs 16.5 ± 0.5 2.7 cekks/gcs in controls; $P < 0.01$), whereas T cell (0.6 ± 0.2 vs 1.1 ± 0.5 cells/gcs in controls: P = ns) and macrophage (3.1 ± 0.8 vx 2.5 ± 0.2 cekks/gcs in controls: P = ns) numbers were unchanged. Treatment had no effect on peripheral white cell numbers. In conclusion, this is the first study to: (1) identify the cell types in which the p38 MAPK pathway is activated in kidney disease, and; (2) demonstrate that functional blockade of the p38 MAPK pathway suppresses renal injury in experimatal glomerulonephritis.

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HEPARANASE IS ASSOCIATED WITH PROTEINURIA IN GLOMERULONEPHRITIS

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Heparan sulphate proteoglycans (HSPG's) are key components of the glomerular basement membrane (GBM), and their glycosaminoglycan (GAG) side-chains contribute to most of its negative charge. Loss of GBM charge has been associated with proteinuria. Heparanase is an endoglycosidase and has, therefore, been considered an important potential contributor to proteinuria through digestion of GAG side chains. To determine the distribution of heparanase in the kidney, a rabbit polyclonal antibody was produced against a 17–amino acid peptide derived from the predicted amino acid sequence of heparanase. The antibody was validated by Western blot analysis of purified recombinant human heparanase. Immunohistochemical staining showed heparanase protein localized to tubular cells in the distal convoluted tubules, thick ascending limb of the loop of Henle, and transitional cell epithelium in normal kidney. Minimal expression was noted in normal glomeruli. In experimental models of minimal change disease, membranous glomerulonephritis and anti-GBM disease, heparanase was up-regulated in glomeruli, predominantly by podocytes. Western blot analysis of lysates from sieved glomeruli showed the processed, active 58 and 52 kDa forms of heparanase, whereas lysates from normal glomeruli contained predominantly the 65 kDa proheparanase species. Northern blot analysis of diseased glomeruli showed increased expression of heparanase mRNA, mainly of the smaller, regulated mRNA species. This data was supported by studies of the transformed human podocyte cell line 56/10A1, which expressed the active form of heparanase. In passive Heymann nephritis, decomplementation with cobra venom factor prevents heterologous phase proteinuria. This maneuvre also prevented the increase in heparanase expression. Heparanase, therefore, is associated with the onset of proteinuria in glomerulonephritis and may contribute to the loss of glomerular charge seen in this condition. Further, heparanase expression appears to be reliant on direct glomerular epithelial cell injury by complement in Heymann nephritis.

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TIMECOURSE OF CORTICAL TUBULAR HYPOXIA IN MURINE ADRIAMYCIN NEPHROSIS

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Background pro-fibrotic effects of chronic tubular hypoxia have been hypothesised as a significant factor contributing to the tubulointerstitial fibrosis which is an inevitable component of chronic renal diseases. The distribution and severity of tubular hypoxia in the face of progressive parenchymal loss is unknown.
Methods we studied cortical tubular hypoxia in murine adriamycin (adr) nephrosis (an)) by quantitative fluorescent detection of the hypoxia marker pentafluorinated etanidazole (ef5). Using computer image analysis, the degree of ef5 binding (hypoxia) was compared between cortical tubules from normal kidneys and those from mice 7, 14 and 28 days following the induction of an.
Results murine an showed characteristic changes of functional impairment and histological damage. Ef5 staining in the normal cortex was most pronounced in the outer medulla, the area of greatest metabolic demand. Within the cortex, there was a small (6% of total) but significant population of tubules demonstrating higher peak ef5 binding rates ($p < 0.05$ vs control) at 7 days after adr, suggestive of patchy relative hypoxia. Despite this, the average level of cortical tubular ef5 binding fell in a stepwise manner after adr ($p < 0.01$ at 14 and 28 days vs control), suggesting an attenuation of tubular metabolic demand. Peak binding rates were not significantly different to control at 14 and 28 days after adr. Overall, average tubular ef5 binding rates corresponded positively with tubular cell height ($r = 0.69$, $p < 0.01$) and inversely with the interstitial volume ($r = -0.66$, $p < 0.01$).
Conclusion These results suggest that tubular hypoxia is not a significant component of advanced murine AN, presumably due to the reduction in oxygen demand associated with parenchymal loss and tubular atrophy. In contrast, patchy focal hypoxia may be present in the early stages of this animal model when histological damage is less prominent, and may potentially contribute to the induction of tubulo-interstititial fibrosis. The pattern of EF5 binding in a murine diabetic nephropathy model is under study.

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RECOMBINANT HUMAN ANTITHROMBIN III HAS PROTECTIVE EFFECTS IN PIG-TO-PRIMATE RENAL XENOTRANSPLANTATION

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Background/Aim. Rejection of pig renal xenografts by primates is associated with intravascular coagulation within the graft and is often accompanied by a form of consumptive coagulopathy in recipients. Using a life-supporting model of pig-to-baboon renal xenotransplantation, we tested the hypothesis that treatment with recombinant human antithrombin III (rhATIII) would prevent or at least delay the onset of these problems.

Methods. Non-immunosuppressed baboons were transplanted with transgenic pig kidneys expressing the human complement regulators CD55 and CD59. Recipients were treated with 8-hourly infusions of rhATIII (250 units/kg), with ($n = 3$) or without ($n = 1$) low molecular weight heparin (LMWH) to maintain supraphysiological levels of ATIII. Regular blood sampling was performed to monitor graft function and parameters of coagulation (platelet count, APTT/INR, D-dimer and fibrinogen).

Results. Circulating ATIII peak and trough levels were 7-fold and 1.2- to 2-fold, respectively, the normal level of $\eta 1$ unit/ml. rhATIII-treated recipients maintained normal renal function for 4 to 5 days, more than twice as long as untreated animals, and developed neither thrombocytopenia nor significantly delayed clotting times during this period. Interestingly, rhATIII did not require LMWH to exert this protective effect. Histological analysis of rejected grafts revealed that rejection was associated with a massive leukocytic infiltration including T cells, macrophages and B cells.

Conclusions. rhATIII may be a useful therapeutic agent to ameliorate both early graft damage and the development of systemic coagulation disorders in pig-to-human xenotransplantation.

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HIGH GLUCOSE EXPOSURE INDUCES ABNORMALITIES IN HUMAN ENDOTHELIAL CELL GROWTH AND DEATH

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High ambient glucose concentrations are known to contribute to diabetic microangiopathy. However the independent effects of the associated hyperosmolar insult are difficult to discern. Hence we have investigated the modes of cell growth and cell death on human endothelial cells exposed to high glucose and an osmotic control.

Experiments were performed in triplicate on 6 different HUVEC isolates and standardised to results obtained with 5 mM glucose. Cell proliferation was measured by cell number whilst cell protein content was measured to assess cell hypertrophy. At 72 hours of 25 mM glucose exposure, a reduced cell number (70.3 +/– 1.97%, $p < 0.0001$) and a parallel increase in cell protein content (142.5 +/– 5.8%, $p < 0.001$) was observed. These effects were less marked with mannitol (the osmotic control) (85 +/– 2%, $p < 0.001$ and 116.3 +/– 6.2%, $p < 0.01$ respectively).

Coincident with the reduction in cell number at 72 hours with 25 mM glucose, a significant increase in apoptosis occurred as measured by morphological staining (152.1 +/– 12.4%, $p < 0.05$)and FACS analysis of preG1 peaks (141 +/– 11.4%, $p < 0.001$) This however was not observed with mannitol which induced cell death mainly by necrosis (158.2 +/– 14.4%, $p < 0.001$) as measured by LDH release with no change in apoptotic markers.

FACS analysis showed that exposure to 25 mM glucose increased the numbers of cells in the S phase of the cell cycle (143.8 +/– 18.8, $p < 0.005$), and also those cells undergoing endoreduplication (170.1 +/– 11.5%, $p < 0.001$).Endoreduplication occurs when DNA synthesis continues in the absence of mitosis. and may increase the cells susceptibility to apoptosis. These effects were not seen with 20 mM mannitol.

In summary, high glucose is associated with a hypertrophic growth response, delay in progression through the S phase of the cell cycle, increased endoreduplication and increased apoptosis. These effects are not observed with mannitol which induces slight endothelial cell hypertrophy and causes cell death by necrosis This study is the first study that glucose has specific effects on cell growth parameters independent of hyperosmolarity.

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ANTI-CD4 mAb THERAPY FACILITATES INDUCTION OF TOLERANCE TO ACTIVE HEYMANN NEPHRITIS BY PRE-IMMUNIZATION WITH RTA/IFA

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Heymann Nephritis (HN) is a rat model of auto-immune mediated human membranous nephropathy. Active HN is induced by immunization of rat renal tubular antigen (RTA) emulsified in complete Freund's adjuvant (CFA). Glomerular cellular infiltrates of rats with HN comprises mainly of CD4+ Th1 cells, CD8+ cytotoxic cells and macrophages. Further more monoclonal antibody (mAb) therapy on T cell subsets have been implicated in the pathogenesis of HN. In this study, the effects of anti-CD4 mAb (OX-38) therapy was examined on induction of tolerance to HN by pre-immunization with RTA in incomplete Freund's adjuvant (IFA) three weeks prior to RTA/CFA immunization. Proteinuria (expressed as mg/100 gm body weight/24 hours) appeared at 8 weeks post-immunization in rats given IFA + anti-CD4 mAb (26.1 ± 4.6) and was similar to HN (27.77 ± 1.6). Proteinuria was absent in RTA/IFA + anti-CD4 mAb (4.25 ± 0.4) and RTA/IFA (7.43 ± 0.8) groups compared to CFA controls (1.26 ± 0.2). A similar pattern was observed at 12 weeks. Serum anti-RTA Ab appeared at 4 weeks in all RTA/CFA immunized rats but not in CFA rats. Titres peaked at 8 weeks in IFA + anti-CD4 mAb group (113.6 ± 13.2%), levels similar to HN controls (92.8 ± 1.9%) but significantly higher than in RTA/IFA + anti-CD4 mAb (40.4 ± 4.0%) or IFA alone (33.7 ± 16.9%). By 12 weeks serum Ab titres declined to levels similar to CFA controls. FACS analysis of popliteal lymph nodes (LN) two weeks after treatment showed 22% depletion of $CD4^+$ cells in RTA/IFA + anti-CD4 mAb compared to RTA/IFA immunized alone. RT-PCR of RTA/IFA + anti-CD4 mAb popliteal LNs revealed a significantly lower level of cytokine mRNA expression for Th1 (IL-2 but not IFN-γ and TNF-β) and higher expression for Th2 (IL-5 and IL-6 but not IL-4 and IL-10) compared to HN controls. This study indicated that $CD4^+$ T cells play a major role in induction of tolerance to HN by immunization with RTA/IFA, and therapy with RTA/IFA further suppressed proteinuria than RTA/IFA immunization alone.

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A DYNAMIC VIEW OF DIALYSIS MODALITY AND SURVIVAL

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Dialysis survival is usually discussed in terms of peritoneal dialysis (PD) compared with haemodialysis (HD), based upon a static view of treatment. We examined the effect of the initial dialysis modality and subsequent switching of dialysis modality on patient survival. We conducted a retrospective analysis using the national data registry for patients who started treatment for ESRD in Australia between 01 Jan 1993 and 31 Dec 1998. We excluded patients from the analysis who died or received transplants before day 90. We defined four different dialysis patterns: P1—HD only, P2—initial HD (at day 90) followed by a switch to terminal PD, P3—initial PD (at or before day 90) followed by a permanent switch to terminal HD, and P4—PD only (this could include up to 2 periods of HD of less than 90 days duration). 262 patients (3.4%) had multiple switches and could not be fitted to the defined patterns. Survival time was calculated from the start of treatment and patients were censored at transplant, loss to follow-up or death. We calculated a hazard ratio for death using the Cox's proportional hazards model with P1 as the reference group. Analysis was adjusted for age, sex, Aboriginality, cause of renal disease, and number of comorbidities at entry to the program. The maximum period of follow-up was 7+ years.

	P1 (HD only)	P2 (HD to PD)	P3 (PD to HD)	P4 (PD only)
Cases (%)	3842 (50.0%)	249 (3.2%)	1057 (13.8%)	2269 (29.6%)
Mortality rate (95% CI) per 100 patient years	12 (11–12)	19 (16–22)	13 (11–14)	22 (21–23)
Adjusted hazard ratio for death (95% CI)	1.00	1.63 (1.34–1.98)	0.96 (0.85–1.07)	1.69 (1.55–1.84)

The patients in groups P2 and P4 were at significantly increased risk of death, while the patients in group P3 had a similar risk of death as the P1 group. This observational study supports the plausible hypothesis that initial PD with a switch to HD to maintain adequate clearance may be an optimal dialysis pattern or at least as good as HD alone. Because treatment choice is influenced by prognosis, we should design RCTs to test this hypothesis.

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A CROSS-OVER TRIAL COMPARING INTRAVENOUS (IV) AND ORAL IRON SUPPLEMENTATION IN ERYTHROPOIETIN (EPO)-TREATED PERITONEAL DIALYSIS (PD) PATIENTS

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Concomitant iron supplementation is required in the great majority of EPO-treated patients with end-stage renal failure. IV iron therapy has been demonstrated to be superior to oral iron therapy in EPO-treated haemodialysis patients, but comparative data in PD patients are lacking. To address this issue, a prospective cross-over study was conducted in PD patients who were on a stable dose of EPO, had no identifiable cause of impaired haemopoiesis and had normal iron stores. Patients received daily oral iron supplements (210 mg elemental iron per day) for 4 months followed by outpatient IV iron infusions (200 mg every 2 months) for 4 months, followed by a further 4 months of oral iron. Twenty-eight individuals were entered into the study and 16 patients completed 12 months of follow-up. Using repeated-measures analysis of variance, haemoglobin concentrations increased significantly during the IV phase (108 ± 3 to 114 ± 3 g/L) compared with each of the oral phases (109 ± 3 to 108 ± 3 g/L and 114 ± 3 to 107 ± 4 g/L, $p < 0.05$). Similar patterns were seen for both percentage transferrin saturation (23.8 ± 2.3 to 30.8 ± 3.0%, 24.8 ± 2.1 to 23.8 ± 2.3, and 30.8 ± 3.0% to 26.8 ± 2.1%, respectively, $p < 0.05$) and ferritin (385 ± 47 to 544 ± 103 µg/L, 317 ± 46 to 385 ± 47 µg/L, 544 ± 103 to 463 ± 50 µg/L, respectively, $p = 0.10$). No significant changes were observed in EPO dosages. IV iron was associated with a much lower incidence of gastrointestinal disturbances (11% versus 46%, $p < 0.05$). In conclusion, 2-monthly IV iron infusions represent a practical alternative to oral iron and can be safely administered to PD patients in an outpatient setting. Compared with daily oral therapy, 2-monthly IV iron supplementation in PD patients was better tolerated and resulted in superior haemoglobin levels and body iron stores.

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VASCULAR DISEASE AND MALNUTRITION IN END-STAGE RENAL FAILURE

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It has recently been suggested that common pathogenic factors contribute to the observed clinical association between malnutrition, chronic inflammation and vascular disease in end-stage renal failure (ESRF). The present study was undertaken to determine the relationship between nutritional measures (total body nitrogen (TBN) and serum albumin) and vascular and infectious complications.

We studied 109 patients who commenced dialysis between October 1993 and September 1998 who had a TBN measurement within 12 months of commencing dialysis (57 HD, 52 PD). Follow up was 37 ± 2.2 months (mean ± SEM). Mortality and morbidity data, including infectious episodes, and ischaemic events in the cardiac, cerebral or peripheral vascular circulation were recorded. TBN was reported as a percentage of that expected for a sex- and height-matched control (Nitrogen Index (NI)). Time to death or first event (vascular or infectious) requiring hospital admission was assessed using the Kaplan-Meier method. NI predicted mortality ($P < 0.05$) and infectious morbidity ($P = 0.02$) but did not predict the development of vascular disease throughout the study period. A serum albumin of less than 35 g/L at the start of dialysis predicted both mortality ($P = 0.004$) and vascular morbidity ($P = 0.02$) over the first three years of dialytic therapy, but not beyond this time point. The NI of patients with ESRF due to renal vascular disease was significantly lower than in those with other causes of ESRF (86 ± 2% vs 94 ± 3%; $P = 0.01$).

We conclude that protein-energy malnutrition does predict mortality and infectious morbidity. However, there is no association between malnutrition and vascular disease. Conversely, the association between hypoalbuminaemia, mortality and vascular disease is strong, but is mediated by factors other than malnutrition.

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THE ATHEROSCLEROSIS AND FOLIC ACID SUPPLEMENTATION TRIAL IN CRF (ASFAST): RATIONALE, DESIGN AND BASELINE RESULTS

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Accelerated atherosclerosis is a major problem in patients with CRF. Premature increase in arterial stiffness also occurs and may be predictive of cardiovascular mortality. The primary objective of ASFAST, a long term multicentre placebo-controlled trial, is to determine if folic acid 15 mg/day slows the progression of carotid artery intima-medial thickness (IMT) in patients with chronic renal failure. Secondary end-points are significant differences between folic acid and placebo treated groups for: (i) clinical cardiovascular events; (ii) arterial function [pulse wave velocity (PWV), aortic pressure augmentation index (A1) and systemic arterial compliance (SAC) and (iii) homocysteine levels. Recruitment commenced on 1.7.1998 and was completed on 31.12.2000, at which time 315 patients were enrolled from 5 renal units (3 Melbourne, 1 Perth, 1 Dunedin, NZ]. Follow-up is planned to 31.12.2003.

Baseline data analysis has been completed. Subjects were aged 24–84 years (mean ± SD, 56 ± 14 years). Sixty one percent were on haemodialysis, 23% on peritoneal dialysis and 16% were predialysis. A history of cardiovascular disease was present in 35%, hypertension in 90%, diabetes in 23%, hypercholesterolaemia in 43% and 14% were current smokers. Hyperhomocysteinemia was present in 97% (27.6 ± 12.9 mmol/l) and correlated inversely with red cell folate and plasma colabamin levels. Biochemical and vascular indices were compared to an age and sex-matched control group (n = 235). Patients with CRF had lower total, LDL and HDL cholesterol levels and higher triglyceride and Lp(a) levels compared to controls. Patients with CRF had higher blood pressure (males 144/82 vs 126/76 mm Hg; females 141/80 vs 119/72 mm Hg), increased IMT (0.85 ± 0.19 vs 0.68 ± 0.11 mm), increased aorto-femoral PWV (10.7 ± 3.5 vs 9.0 ± 0.68), increased aorto-femoral PWV (10.7 ± 3.5 vs 9.0 ± 1.7 m/sec), increased AI (males 21 ± 12% vs 15.3 ± 1%, females 25 ± 11% vs 23 ± 0.8%) and reduced SAC (0.44 ± 0.23 vs 0.59 ± 0.21 units). Examination of the age-related changes in IMT and arterial stiffness (PWV) showed a marked shift to the right in the CRF group compared to controls. To date, in 400 person-years of follow-up, there have been 19 cardiovascular events and 25 deaths.

These results indicate the need for better blood pressure control in this population, a very high risk group for cardiovascular events. This population exhibited a 15–20year shift to the right in age-related changes in IMT and arterial stiffness. The ASFAST study is powered to provide a definitive answer to the question as to whether or not folate therapy has cardiovascular benefits in the CRF population.

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INDUCIBLE EXPRESSION OF CONNECTIVE TISSUE GROWTH FACTOR BY CULTURED HUMAN PERITONEAL MESOTHELIAL CELLS

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Time on peritoneal dialysis is associated with progressive damage and fibrosis of the peritoneal membrane. The fibrogenic cytokine transforming growth factor-beta (TGFbeta) is present in the peritoneal cavity and has been implicated in this fibrotic response. TGFbeta has pleiotropic effects, and recent evidence suggests that its role in fibrosis is mediated by a more specific downstream cytokine known as connective tissue growth factor (CTGF). We therefore sought to determine whether CTGF was produced in the peritoneal cavity of peritoneal dialysis patients. Peritoneal cells were isolated from discarded dialysate, and human peritoneal mesothelial cells (HPMC) isolated and cultured according to our published method. CTGF mRNA was readily detectable by RT-PCR in unfractionated peritoneal cells from multiple different patients. Northern blotting of mRNA from cultured HPMC showed 2.4 and 1.45 kb bands consistent with CTGF and TGFbeta, respectively. Culture of HPMC in the presence of TGFbeta (2 ng/ml) induced the CTGF message. Western blotting with a CTGF-specific polyclonal antibody revealed bands consistent with CTGF protein in the culture supernatant of HPMC. This is the first report of CTGF expression by HPMC and its induction by TGF in these cells, and may indicate a key pathway for development of fibrosis in the peritoneal cavity.

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LONG TERM HAEMODIALYSIS ACCESS: FACTORS INFLUENCING CHOICE AND SURVIVAL

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A retrospective review was undertaken in the prevalent haemodialysis population of an Australian Tertiary Renal Unit of patients who started haemodialysis between April 1994 and September 2000.

The study aimed to assess the factors affecting the choice of long-term arteriovenous haemodialysis access and the factors influencing access survival. Data were obtained from clinical charts and ANZDATA Registry data. Minimum follow up was 3 months. Results: n = 71. 66.2% male, 22.5% diabetic, mean age 56.9 yrs. Late referral (< 3 months) 35.2%. Only 39% of patients were Australian born. 71 patients had a total of 96 new vascular access operations and 57 revision operations.

Of 71 first access procedures 56 were native fistulae and 9 were synthetic grafts. Only 31% of long-term access was in place 6 weeks or longer prior to start of dialysis. Of 25 subsequent (new) access placements only 8 were fistulae and 17 were grafts. Diabetes, peripheral vascular disease and coronary artery disease all significantly predisposed to placement of synthetic grafts. First native fistulae had superior survival to subsequent native fistulae. Subsequent fistulae had a significant rate of early loss, but were then stable. Synthetic graft survival was similar whether first or subsequent access, and was always inferior to native fistulae (p = 0.0072).

Although native fistulae are already favoured for first access these results suggest that native fistulae should more often be considered for subsequent (secondary) access. Access survival of synthetic grafts vs native fistulae is presented below.

ASSISTED PATENCY

		1 YEAR	2 YEAR	3 YEAR	4 YEAR
AV FISTULA	FIRST	94%	94%	94%	78%
AV FISTULA	SUBSEQUENT	73%	73%	73%	72%
SYNTH GRAFT	FIRST	70%	70%	50%	50%
SYNTH GRAFT	SUBSEQUENT	68%	50%	50%	50%

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DOES COMBINED BLOCKADE OF THE RAS AND AGE FORMATION CONFER SUPERIOR RETROPROTECTION IN A HYPERTENSIVE MODEL OF DIABETIC NEPHROPATHY?

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Clinical studies indicate that the major determinants of diabetic nephropathy are hyperglycemia and systemic hypertension. Although experimental studies have explored the role of antihypertensive treatment, in particular the blockade of the RAS and the effect of inhibition of AGE (advanced glycation endproduct) formation, no studies have assessed if these two treatments are synergistic in conferring renal protection in diabetes. Streptozocin diabetic rats with concomitant hypertension (SHR) were randomized to receive either no treatment (n = 14), the ACE inhibitor perindopril (PER 2 mg/L, n = 12), an inhibitor of AGE formation, aminoguanidine (AG 1 g/L, n = 16), or the combination of AG and PER (n = 12). Non-diabetic SHR served as controls. Data are shown at week 20 as means for systolic blood pressure and geometric means for albumin excretion rate (AER).

Diabetes was associated with a modest lowering of SBP (control 204 ± 3 vs diabetic 194 ± 5 mmHg, P < 0.01) which was further reduced by PER (151 ± 3 mmHg, p < 0.01 vs diabetic) or the combination PER/AG (165 ± 3 mmHg, p < 0.01 vs diabetic) but not by AG alone (182 ± 5 mmHg). In diabetic animals there was a progressive increase in AER at week 20 (61, p < 0.01 vs control, 2 mg/24 hr) whilst each monotherapy blunted the rise (PER or AG, 20 mg/24 hr). However, a further significant reduction in AER occurred using the combination PER/AG (11 mg/24 hr, p < 0.01 vs PER and AG alone). The present study indicates that both ACE inhibition and inhibition of AGE formation attenuates development of albuminuria in diabetic SHR. It is postulated that combination therapy that blocks both hemodynamic and metabolic pathways may provide superior renoprotection in a model of diabetes and hypertension.

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PARATHYROID HORMONE HAS A PROSCLEROTIC EFFECT ON VASCULAR SMOOTH MUSCLE CELLS

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Although accelerated atherosclerosis and arteriosclerosis are common in patients with renal failure, the pathogenesis of these changes is poorly understood. Rat data suggests that the vascular smooth muscle cell (VSMC) contributes to these abnormalities through increased growth and matrix production. Parathyroid hormone (PTH) levels are elevated in renal failure, and these elevated levels have been linked to uraemic vascular changes in some studies. To investigate whether hyperparathyroidism contributes to the vascular changes of uraemia, we examined the in vitro effects of increasing doses of the 1–34 fragment of PTH on the growth and function of human aortic vascular smooth muscle cells (VSMC, Clonetics, USA). All results are expressed as % of control and compared by ANOVA.

At 200 pmol/L, PTH increased total collagen synthesis as measured by tritiated hydroxyproline incorporation (188 ± 25%, $p < 0.01$) and the expression of α1(I) procollagen mRNA (136 ± 11%, $p < 0.05$). PTH also increased reorganisation of collagen I lattices as measured by reduction in diameter at 100 and 200 pmol/L (76 ± 5% and 73 ± 6% respectively, $p < 0.05$), and upregulated expression of β1 integrin mRNA, a cell surface molecule involved in collagen reorganisation (163 ± 20% at 200 pmol/L, $p < 0.05$). PTH had no effect on VSMC proliferation (96 ± 7%, p = NS) or cell number after 5 days growth (97 ± 10%).

As collagen production and reorganisation are the hallmarks of sclerosis, these results suggest elevated concentrations of PTH have a prosclerotic effect on VSMCs at serum concentrations similar to those observed in many uraemic patients. This effect may contribute to the accelerated vascular disease and increased cardiovascular mortality and morbidity seen in this patient group.

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ATORVASTATIN'S ROLE IN MATRIX PRODUCTION IN HUMAN ENDOTHELIAL CELLS

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The statin family of dugs have been proven to reduce the vascular complications associated with atherosclerosis by lowering cholesterol and affecting cytokine and cell matrix production. Since high glucose is a risk factor for the development of vascular plaques which contain excess matrix, we have investigated the effects of atorvastatin on matrix production by human endothelial cells exposed to both normal and high glucose conditions.

Human umbilical vein endothelial cells (HUVECs) were exposed to glucose specific media in the presence or absence of 1 μmol/L atorvastatin. Collagen synthesis was measured by ^{3}H-proline uptake. Matrix metalloproteinase 9 (MMP9) and its opposing enzyme tissue inhibitor of metalloproteinase (TIMP1) were measured using gelatin zymography and western blot respectively on cell supematant.

Exposure of 25 mM glucose over 72 hours was associated with a significant increase in TIMP 1 production (140.9 + 9.4%; $p < 0.001$), no significant increase in MMP 9 release and a trend towards reduced ^{3}H-proline uptake (81.9 + 5.3%). Exposure of 1 μmol/L atorvastatin in 5 mM glucose was associated with a significant increase in MMP 9 production (138.1 ± 9.8%) or ^{3}H-proline uptake (87.8% ± 12.8%). Exposure of 1 μmol/L atorvastatin in 25 mM glucose was also associated with an increase in MMP9 release (145.8 ± 7.5%; $p < 0.0001$), reduced ^{3}H-proline uptake (70.7% ± 10.4%) and no significant effect on TIMP1 release (117.1 ± 12.1%).

In summary, 25 mM glucose exposure to HUYVECs is associated with an increased TIMP1/MMP9 ration enhancing matrix production. Atorvastatin reduced this ratio hence reducing matrix deposition. These effects of atorvastatin may lead to reduced atherosclerotic plaque size and reduced glomerular basement membrane thickening associated with diabetic nephropathy.

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LDL MODULATES GROWTH RESPONSES IN HUMAN PROXIMAL TUBULAR CELLS (PTC)

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Abnormalities of lipid metabolism, with increased low density lipoproteins (LDL), are seen in renal disease, particularly when proteinuria is present. The proximal tubule is the primary site of LDL uptake and metabolism in the kidney. The aim of this study was to determine the direct effects of LDL on growth responses of PTC in the presence and absence of albumin. Primary cultures of human PTC grown in serum free medium were exposed to native LDL (10 and 100 ug/ml) and/or albumin (1 mg/ml). Cell number and thymidine incorporation (proliferation), and protein content (hypertrophy) were measured and reported as a percentage of results obtained in the absence of LDL and albumin. LDL oxidation (consumption of alpha-tocopherol and generation of cholesteryl ester hydroperoxides and hydroxides (CEO(O)H)), was measured by HPLC. Cells exposed to 10 ug/ml LDL for 48 h showed a hypertrophic response (120 ± 7%; mean ± SEM; $P < 0.005$), with no change in cell number or thymidine incorporation. By 72 h, an increase in cell number was evident (112 ± 6%; $P < 0.05$). Cells exposed to 100 mg/ml LDL showed an increase in protein content at 48 h (118 ± 6%; $P < 0.01$). At 72 h this translated into marked decreases in both cell number (80 ± 3%; $P < 0.0001$) and thymidine incorporation (60 ± 3%; $P < 0.0001$), suggestive of a block in cell cycle progression. The hypertrophic effects of both 10 and 100 ug/ml LDL, and subsequent inhibition of cell division in the presence of 100 ug/ml LDL, were abolished in the presence of 1 mg/ml albumin. At 72 h, there was a low level of spontaneous oxidation of LDL (complete loss of alpha-tocopherol and a marginal increase in CEO(O)H). There was no inhibition of oxidation in the presence of albumin. These data show that in PTC, low levels of LDL stimulate cell growth, whereas high concentrations inhibit the proliferative response. The growth inhibitory effects of high LDL correlate with the formation of partially oxidized LDL. However, the cytoprotective effect of albumin in the presence of LDL is independent of the oxidation state of LDL.

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MODULATION OF HUMAN PROXIMAL TUBULE RESPONSES TO ANGIOTENSIN II (AII) BY HIGH GLUCOSE

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It is well established that the tubulointerstitium of the rat kidney synthesises all components of the renin-angiotensin system. However, the local action and functional regulation of AII in the human kidney is not known. The present study investigates the potential autocrine effects of AII on human proximal tubular cells (PTCs) and the effects of concomitant exposure to high glucose on AII related growth and transport activity.

Primary cultures of PTCs from at least 6 individuals were exposed to either AII and/or high (25 mM) glucose. Standard PCR techniques identified the AT1A receptor. ELISA was used to measure AII production. ^{22}Na uptake was used as a measure of Na-H exchange (NHE) activity. cAMP was measured in culture supernatants by RIA. Growth parameters including cell number and thymidine incorporation and protein content were expressed as a percentage of results obtained in 5 mM glucose.

We found that primary cultures of PTCs produce AII. Exposure of PTCs to AII resulted in an increase in ^{22}Na uptake at low concentrations (119.2 +— 8.9% at 10^{-10} M ($P < 0.05$) and 123.7 +— 10.8% at 10^{-8} M ($P < 0.01$) and a decrease at high concentrations (72.0 +— 4.8% at 10^{-6} M ($P < 0.001$). These effects were due respectively to stimulation and inhibition of NHE3 activity. No change was observed in cAMP production by PTCs in response to AII, suggesting that the action of AII is independent of cAMP. Exposure to 10^{-8} M AII for 48 h led to an increase in cell number (114.1 +— 4.3%, $P < 0.01$). In contrast, when cells are exposed to AII and high glucose, the proliferative response was abolished and a hypertrophic response was observed.

These data are consistent with a role for autocrine aii in the control of cell growth and na transport in the proximal tubule. The hypertrophic effect and enhanced na reabsorption seen with high glucose and elevated aii levels presents a potential mechanism linking the functional and pathological abnormalities observed in diabetic nephropathy.

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FAS DEPENDENT APOPTOSIS CONTRIBUTES TO TUBULAR ATROPHY IN EXPERIMENTAL DIABETIC NEPHROPATHY: EFFECTS OF ACE INHIBITION

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Renal tubular atrophy, mostly a consequence of apoptosis, predicts a poor prognosis in chronic renal disease. We hypothesized that Fas (CD95) death receptor, not normally present in the kidney, may become aberrantly expressed by the tubular epithelium in diabetic nephropathy (DN) and that its modulation may contribute to the renoprotective effects of ACE inhibition. Streptozotocin diabetes was induced in the Ren-2 rat, a model which when rendered diabetic develops the structural and functional features of human DN. Rats were randomized to 3 group (n = 6/group): control (C), diabetic (D), diabetic treated with perindopril (DP) and examined at 12 weeks by quantitative in situ hybridisation, immunohistochemistry and TUNEL.

	Fas (P/A)	ssDNA	TUNEL	Tub. atrophy (P/A)
C	0.03 ± 0.03	1 ± 1	2 ± 1	0
D	2.67 ± 0.52*	50 ± 10**	64 ± 11**	10.2 ± 1.1**
D + P	0.98 ± 0.36†	15 ± 4†	25 ± 9†	2.1 ± 1.0†

* $p < 0.05$, ** $p < 0.01$/vs C. † $p < 0.01$ v D. P/A proportional area/tissue section. ssDNA and TUNEL expressed as cells/3–5 tissue sections.

Only occasional ssDNA or TUNEL + ve cells and no Fas + ve cells were detected in control Ren-2 rats. In diabetic Ren-2 rats, however, de novo expression of Fas by tubular epithelial cells was noted with tubular apoptosis and atrophy. All changes were attenuated by perindopril. **Conclusion:** Fas-mediated apoptosis may contribute to tubular atrophy in experimental DN and its attenuation may underlie the renoprotective effects of ACE inhibition.

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LIVE DONOR RENAL TRANSPLANTATION IN AUSTRALIA 1964–99—AN EVOLVING PRACTICE

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Australia has a low organ donor rate (9.0/10^6 in 1999) compared to other developed countries (USA—21.4/10^6 in 1999), leading to the greater use of live donor (LD) grafts. This study reviewed the Australian experience with LD transplants from 1964–99. Data for all LD and cadaveric (CD) transplants was obtained from the Australian and New Zealand Dialysis and Transplant registry (ANZDATA). Survival was assessed by the Kaplan-Meier method. The log-rank test was used to determine statistical significance between survival curves. 1584 LD and 10 252 CD transplants were performed between 1964–99. While the CD rate dropped over the last decade, the LD rate increased, maintaining the overall transplantation rate. Only 3.6% of grafts before 1980 were LD, increasing to 28.4% during 1995–99. Patient and graft survival of LD grafts was superior to CD grafts. Most LD grafts (90.3%) were from live related donors (LRD), most commonly parents (43.2%) or siblings (38.9%). Transplants from live unrelated donors (LURD) dramatically rose (1980–89 n = 6, 1990–99 n = 143), primarily due to more spousal donation, with no significant difference in survival between LRD and LURD groups. Most LD recipients were aged 20–49 (65%). Recipients over age 50 increased from 7.6% in 1981–90 to 16% in 1991–99, and grafts from older donors (> 50) also increased (1980–89—14.4%, 1990–99—28.6%). Between 1980–99 there was no graft survival difference between donors < 50 and donors > 50. LD transplants prior to commencement of dialysis rose from 8.1% in 1981–90 to 15.6% in 1991–99. From 1964–99, 201 predialysis transplants were performed, with survival similar to grafts in the dialysis group. In summary, the pattern of LD transplantation in Australia has changed markedly, with more unrelated and older donors, and more pre-emptive transplants. Long-term patient and graft survival advantages have been maintained, supporting the growing use of live donors to expand the donor pool.

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RENAL ALLOGRAFT LOSS IN THE AUSTRALIAN PAEDIATRIC POPULATION

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Children are at highest risk of graft loss of any subgroup undergoing renal transplantation. Relevant ANZDATA registry data was accessed for the period 1989 to 1998 to determine actuarial allograft survival in recipients up to 20 years of age [n = 180]. Recipient and donor factors likely to impact on graft loss were also examined. The Kaplan-Mayer method was used to estimate actuarial survival. Primary renal disease included reflux nephropathy [n = 31], congenital renal hypoplasia or dysplasia [n = 24], glomerulonephritis other than focal segmental glomerulosclerosis [n = 23], posterior urethral valves [n = 21], medullary cystic disease [n = 14], metabolic disorders [cystinosis n = 11, oxalosis n = 2], focal segmental glomerulosclerosis [n = 11], haemolytic uraemic syndrome [n = 9], lower urinary tract abnormalities other than posterior urethral valves [n = 9], infantile or juvenile polycystic kidney disease [n = 5] and miscellaneous [n = 20]. Graft loss was seen in 29 patients, with a cumulative incidence of 11.2% [95% CI: 7.4%, 16.8%] by one year and 17.4% [95% CI: 11.6%, 25.6%] by five years post-transplant. The most common causes of graft loss were chronic rejection [n = 8], acute rejection [n = 7] and vascular complications [renal artery thrombosis n = 6, renal vein thrombosis n = 1]. Primary renal diagnosis [focal segmental glomerulosclerosis, haemolytic uraemic syndrome and lower urinary tract abnormalities other than posterior urethral valves], recipient age [less than 5 years] and donor age [less than 10 years] increased the risk of graft loss. Other factors [recipient or donor sex, time on dialysis pre-transplant, panel reactive antibody levels, organ source, number of HLA mismatches and cold ischaemia time] were not predictive of graft loss.

	age, years		months to graft failure	
no graft failure, n = 151	14.7	[3.0–19.9]		
graft failure, n = 29	13.1	[3.4–20.0]	0.6	[0.0–109.6]
chronic rejection, n = 8	13.0	[4.1–18.6]	51.2	[8.3–109.6]
acute rejection, n = 7	7.2	[3.4–19.8]	0.6	[0.0–4.9]
vascular complications, n = 7	13.9	[11.2–17.6]	0.1	[0.0–0.2]
technical complications, n = 3	18.6	[9.0–19.3]	0.0	[0.0–0.3]
recurrent glomerulonephritis, n = 2	19.2	[18.3–20.0]	35.7	[0.7–70.7]
malignancy, n = 2	13.0	[10.9–15.1]	7.8	[0.0–15.6]

median [range]

P76

ASSOCIATION OF CYCLOSPORIN SPARING AGENT USE WITH GRAFT AND PATIENT OUTCOME

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Use of cyclosporin sparing agents (CsSA) in renal transplantation is widely practiced in Australia and NZ. Introduced to costs of cyclosporin, an independent effect on outcome has also been suggested. We analysed the relationship between use of these agents and transplant outcome using data from ANZDATA Registry.

Outcome (patient death or graft loss) for 1st grafts in centres performing > 25 transplants/year was compared between CsSA and non-CsSA use, and also CsSA users (Group 1) and non-users (Group 2)in 'routine' CsSA (centres where > 25% of all grafts receive CsSA) and recipients in 'non-routine' CsSA centres (Group 3).

Between 1 Jan 1993 and 1 Sep 2000, 2286 grafts (72% CD1, 28% LD1) were performed and 62% given a CsSA from time of operation. There were no significant differences in live donor outcomes between the groups. There were significant differences in age, diabetes (DM) prevalence, donor age and DR matching between the groups. There was no association between CsSA and rejection rates in the first 6 months. LD1 were more likely than CD1 to receive CsSA (OR 1.3 [1.0–1.6]).

Although overall CsSA use was associated with improved CD1 survival (HR 0.76 [0.61–0.96]), crude survival did not differ between groups 1 and 3 (HR 1.1 [0.8–1.3]) but was poorer in group 2 (HR [1.6 [1.2–2.0]). After adjustment for covariates this difference was less significant (HR 1.3 [1.0–1.8]). Rates of acute rejection (< 6 months) did not differ between groups. There was a trend to less DGF in the group 1 compared to group2 (HR 1.5 [1.1–2.0]) and 3 (HR 1.7 [1.4–2.1]).

Use of CsSA is widespread in Australia and New Zealand. CsSA use is associated with a graft survival advantage and reduced DGF on crude analysis, but when 'routine' CsSA centres are compared to 'non-routine' the survival difference is of marginal significance. Within 'routine' centres outcomes differ with CsSA use. There are unexplained differences in co-variates between the groups. Although statistically adjusted, the effect might be due to residual confounding by unmeasured factors or selection bias, and is limited by the validity of available co-morbid information.

P77

MONITORING OF CYCLOSPORIN IN STABLE RENAL ALLOGRAFTS: C_0 OR C_2?

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Background: Trough levels (C_0) are traditionally used for monitoring of Cyclosporin in renal allograft recipients. C_2 levels have been found to better correlate with area under the curve (AUC_{0-12}), likelihood of rejection and nephrotoxicity in the early months post transplant. A therapeutic range for C_2 at day 180+ of 500–1100 g/L has been suggested but not validated. We have undertaken absorption profiling of CSA in stable transplant recipients (>2 years) to examine: (1) correlations between C_0, C_2, AUC, absorber status and GFR, (2) comparisons between our currently targeted C_0 range (100–200 gL) and the recommended range for C_2 (500–1100 g/L), (3) the implications for CSA dosing of changing to C_2 monitoring. Methods: 67 renal allograft recipients attended for supervised blood sampling at 0, 2 and 3 hours post dose and CSA levels measured by EMIT assay. AUC, GFR, absorber status and required dose adjustments were calculated using published formulae. Results: Mean C_0=154 (±65), C_2=779 (±259), AUC_{12}=4130 (±1404). Correlations of C_0 vs C_2 (r=0.66) and C_0 vs AUC (r=0.875) were better than studies in early transplants have indicated. GFR did not correlate with any measured parameter. 40% of patients were good absorbers, 19% were poor absorbers. 45% were outside C_0 targets (18%>100, 20%>200). 27% were outside C_2 targets (13%<500, 14%>1100). Dose change to target C_0=150 was +23 mg/pt/d and C_2=800 was +21 mg/pt/d.
Conclusions: Compared to published data from studies in recent transplants, long term patients are better absorbers of CSA and have C_0 levels which correlate better with C2 and AUC_{0-12}. Based on C_0 levels 45% of our patients require dose adjustment. If C_2 monitoring was adopted 55% of those changes would be inappropriate. If current C_2 guidelines are clinically validated dosing decisions made on the basis of C_0 levels would be wrong in about one third of cases. Whether C_0 or C monitoring is used an average dose increment of just over 20 mg/day was required to target mid-range levels.

P79

PERIOPERATIVE HYPERTENSION AND ACUTE REJECTION FOLLOWING TRANSPLANTATION

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Background: According to the 'injury response' hypothesis, acute rejection may be initiated or amplified in stressed or injured tissue. Shear stress up-regulates adhesion molecules, cytokines and antigen presentation may potentiate reperfusion injury. Auto-regulation in a denervated allograft is also impaired. Early exposure to hypertension might therefore influence the alloimmune response.

Methods: CD1 pateints receiving cyclosporin-based therapy were reviewed (n=276). Blood pressure readings were obtained from clamp-release to 50 hours after surgery. Mean exposure was estimated from the area under the curve (AUC). Variables predicting rejection were identified using a logistic regression model.

Results: The mean (SD) blood pressures were 161(19) mmHg systolic and 73(12) hypertension as defined by conventional parameters. Acute rejection occurred in 59% of patients. Systolic pressure exposure was associated with an increased risk of rejection (table). Neither mean nor diastolic pressures were predictive. The median time to rejection was 12 days, with hypertensive patients having earlier rejection. Hypertension was independent to known risks for rejection and occurred must earlier, suggesting hypertension was not the result of occult rejection. Low pressures had no effect on early graft function but reduced the risk of rejection.

Conclusions: Hypertension is common following renal transplant surgery. We propose that early hypertension may contribute to an excess of rejection episodes.

Rejection	Odds Ratio	Standard error	P>[z]	95% Confidence	Interval
Age	.9703638	.0112612	0.010	.9485414	.9926882
Triple therapy (vs double)	.3275896	.111675	0.001	.1679408	.6390046
Delayed graft function	.464518	.147796	0.016	.2489861	.8666226
Systolic pressure AUC	1.015751	.0077929	0.042	1.000592	1.031141
HLA matching	.7536438	.0757691	0.005	.618855	.9177901
Mycophenolate (vs aza)	.3135862	.0974837	0.000	.1705085	.5767236

Model parameters: Area under ROC curve=0.7715; Hosmer-Lemeshow x^2=6.25.

P78

EARLY RENAL TRANSPLANT OUTCOMES IN AUSTRALIAN ABORIGINES

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BACKGROUND: Long-term transplant outcomes are impaired in Aboriginal patients. It is not clear whether this represents late morbidity or the consequence of early graft injury. We review the first months care of Aboriginal patients transplanted in a single center (n=109) and compare non-Aboriginal patients transplanted at the same time.
DEMOGRAPHICS: The mean (SD) age of Aboriginal patients was 43 (11) years, half were men. Renal failure was attributed to diabetes (35%) or glomerulonephritis (50%) or was of unknown etiology (18%). 62% had diabetes and 30% were obese (BMI>30).
TRANSPLANT: Aboriginal patients were more likely to be receiving their first graft from a cadaveric source (CD1). HLA matching was worse and mismatching greater than in non-Aboriginal patients. Ischaemic times were longer for Aboriginal CD1s (20.4 vs 15.4, p<0.01) reflecting travel distances (some over 3000 km). The duration of operation was also longer for Aboriginal patients who received greater volume resuscitation and were more likely to require transfusion during surgery. 96% of Aboriginal patients received cyclosporin-based therapy (70% non-Aboriginal patients). Prednisolone, mycophenolate and azathioprine were used to a similar extent.
OUTCOMES: 40% of Aboriginal CD1 patients had delayed graft function requiring dialysis. Race was an independent predictor for delayed graft function on multivariate analysis. 50% had an acute rejection episode, requiring monoclonal therapy in 12%. 84% of Aboriginal patients had total cyclosporin dose reductions of >25%, compared to 23% of non-Aboriginal patients, possibly reflecting pharmacokinetic differences. Aboriginal patients were more likely to have impaired graft function at one month.

CD1Outcome	Aboriginal	Non-Aboriginal	Significance
Delayed graft function	40%	20%	<0.01
Acute Rejection	50%	55%	NS
Graft function at one month			
Non-function	10%	6%	<0.01
GFR 20–50 ml/min	41%	26%	0.02
Mean GFR (functioning)	43 ml/min	54 ml/min	0.05

P80

OUTCOMES WITH RENAL TRANSPLANTATION IN INDIGENOUS AUSTRALIANS

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Indigenous Australians have a nine times higher standardised incidence of ESRD than the total Australian population. Previous research has indicated poor outcomes with dialysis and transplantation. As part of a study examining barriers to access and predictors of outcome, we conducted a retrospective analysis using the Australia and New Zealand Dialysis and Transplant Registry for 8128 patients (719 Indigenous and 7409 non-Indigenous) who started treatment for ESRD in Australia between 01 Jan 1993 and 31 Dec 1998. We analysed the likelihood of receiving a transplant, delay to transplant, and patient and graft survival after first transplant, for Indigenous and non-Indigenous patients, using the Cox's proportional hazards model. Analysis was adjusted for age, sex, cause of renal disease, and number of comorbidities at entry to the program. Post-transplant survival was also adjusted for the time to transplant (from starting dialysis). The maximum period of follow-up was 7 years. 78 (10.9%) Indigenous patients and 1834 (24.8%) non-Indigenous patients received transplants in this period. Indigenous patients were less likely to receive a transplant (adjusted rate ratio 0.34, 95% CI 0.27–0.43). The median time to transplant was 1.23 years for non-Indigenous patients and 1.99 years for Indigenous patients. Indigenous patients were at significantly increased risk of death post transplant, but this relationship was attenuated after adjustment for age, time to transplant, sex and number of comorbidities (adjusted hazard ratio 1.72, 95% CI 0.88–3.38). Although patient and graft survival were lower for Indigenous patients, survival of Indigenous patients has improved from previous reports. Indigenous patients continue to have significantly reduced access to renal transplantation and increased delay for those who receive a transplant. We need to identify barriers to Indigenous patients accessing transplant services and work to further reduce the gap in survival.

P81

CUMULATIVE SURVIVAL RATE BETWEEN ESRD PATIENTS UNDER TREATMENT HEMODIALYSIS AND KIDNEY TRANSPLANTATION

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Both chronic hemodialysis and renal transplantation as definitive treatment of End Stage Renal Disease are very-expensive. Within the last 2 years, the number of hemodialysis machines in Hemodialysis Unit Dr Soetomo Hospital has been added into 20 machines. Until now, the renal transplantation in Surabaya are only from living related donors, and the limited number of donors are our main limitation. We would like to compare the survival rate of ESRD patients treated with hemodialysis and those with renal transplantation from overseas with cadaveric donor. The data were taken from all ESRD patients under chronic hemodialysis at Hemodialysis Unit Dr Soetomo Hospital or having renal transplantation from 1998 to 2000. In our hemodialysis unit, from the year 1998, 1999 and 2000 there were 48, 118 and 129 patients respectively. Of 98 renal transplanted patients, 70 patients had overseas renal transplantation. Using Cumulative Survival of Kaplan Meier, the results are:

Cummulative Survival Rate	KIDNEY TRANSPLANT (n=70)	HEMODIALYSIS 1998 (n=48)	1999 (n=118)	2000 (n=129)
1st year	94%	62,5%	82,1%	84,2%
2nd years	76,36%	60,5%	76,2%	—
3rd years	58,35%	59,6%	—	—

The survival rate of ESRD patients increased with addition of hemodialysis machines. The data above showed that renal transplantation is still a better choice of treatment in ESRD patients than hemodialysis. Management of ESRD patients should be directed for renal transplantation.

P82

TRANSPLANT PATIENT FITNESS ASSESSMENT: SUBJECTIVE & QUANTITATIVE CRITERIA ANALYSIS

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Categorical patient fitness (A good, B moderate, C poor, D unacceptable) has an impact on graft survival equivalent to that of HLA matching, in both Collaborative Transplant Study (CTS) and Western Australian data sets. To determine whether subjective criteria (SC) grading of patient fitness was consistently applied between adult nephrologists, arbitrary quantitative criteria (QC) were applied for age, cardiovascular disease, body mass index, compliance, smoking, carcinoma, infection and other organ dysfunction. All patients on the transplant waiting list in Perth (n=109) were given a concurrent subjective and quantitative grading by their supervising clinician, with overall ranking determined by lowest ranking in any category.

		Subjective Criteria %				Quantitative Criteria %			
Hosp	n	A	B	C	D	A	B	C	D
1	40	35	45	17.5	2.5	22	50	22	5
2	51	61	24	12	4	16	35	39	10
3	18	61	22	5	12	55	28	5	12

There was broad agreement between SC & QC, except in Category A at Hospital 2. 38% of all patients were downgraded by QC compared to SC; the common reasons were smoking (18%), compliance (16%), BMI (14%), cardiovascular disease (9%) and age (9%). In 5 hypothetical WA organ allocations, the top-ranked recipients by HLA-matching and waiting time were QC Category B in 3 cases and C in 2 cases. Using CTS mean Tx survivals, this represents a mean loss of 37 months per graft, compared to expected graft survival for adjacent category A recipients. This study supports the use of QC to improve inter-observer consistency in Tx recipient fitness, QC may assist in assessing patient suitability to be placed on a transplant waiting list.

P83

NEUTROPHIL DYSPLASIA CHARACTERIZED BY THE ACQUIRED PELGER-HUET ANOMALY OCCURRING WITH THE COMBINATION OF MYCOPHENOLATE MOFETIL AND GANCICLOVIR POST RENAL TRANSPLANTATION: A REPORT OF 5 CASES

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The acquired Pelger-Huet anomaly (APHA) has been associated with a variety of primary haematological disorders, infections and drugs. We report 5 cases of dysgranulopoiesis characterized by the APHA occurring with the combined use of mycophenolate mofetil (MMF) and ganciclovir post renal transplantation. This developed a mean of 118 days (range 66 to 196 days) after transplantation, and resolved within 91 days (range 26 to 175 days) as a result of cessation and/or dose reduction of both of these drugs. In 3 cases significant neutropenia also occurred, which appeared to be correlated with the percentage of circulating neutrophils displaying dysplastic morphology. We recommend that all patients receiving MMF and/or ganciclovir post renal transplantation have regular peripheral blood films reviewed. If dysplastic granulopoiesis develops, dose reduction and/or cessation of both MMF and ganciclovir should be considered until resolution of the dysgranulopoiesis occurs.

P84

VASCULAR STAINING FOR IgM, C3, & C1q IN BORDERLINE & ACUTE CELLULAR REJECTION DOES NOT CORRELATE WITH GRAFT OUTCOME

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Endothelial and transmural staining of arteries & arterioles for IgM, C3 & C1q in renal allografts is a key finding in acute vascular rejection (VR). Given the worst prognosis of VR we hypothesised that significant arteriolar &/or arterial staining for IgM, C3 & C1q in patients with cellular rejection (CR) may indicate either early VR or concomittent VR missed by sampling error, and therefore may be a predictor of e poor patient outcome. We retrospectively examined the relationship between vascular IgM, C1q, C3 and graft outcome in patients with CR. Transplant recipients (46 kidney, 9 kidney-pancreas) with biopsy proven acute rejection (borderline changes, Ia or Ib rejection, Banff '97) were studied. Parrifin sections were stained for IgM, C3, C1q and graded 0=no staining, 1=faint/mild, 2=moderate, 3=heavy by a pathologist blinded to patient outcome and biopsy diagnosis. Interim analysis for each stain showed no correlation between grade of staining and graft function at one year each of the 3 individual stains. A combined score was then assigned to each biopsy (0–3 points for each stain IgM, C3, & C1q, total 0–9). Comparison of those with minimal (score 0–2 group 1, n=21) versus significant (score 3–9 group 2, n=34) staining by survival analysis was performed, using end-points of serum creatinine ≥200 μmol/L within 12 months, serum creatinine ≥200 μmol/L at last follow-up and graft loss in separate analysis. No significant difference between the groups was evident for any end-point, however 4 grafts were lost to rejection in group 2 versus none in group 1 (group 1 : 0/21 0% 95% C.I.=0–16% vs group 2: 4/34 C.I.=3.0–27.5% p=0.286). Vascular deposition of IgM, C1q, C3 in cases of borderline and CR is not a significant predictor of outcome, however graft loss due to rejection was not seen in subjects with minimal deposition.

P85

PARTIAL DEPLETION OF MACROPHAGES BY ED7 REDUCES RENAL INJURY IN ADRIAMYCIN NEPHROPATHY

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Macrophage infiltration has been demonstrated in various types of glomerulonephritis, incldng focal glomerulosclerosis. However, the role of macrophages in focal glomerulosclerosis is still unclear. A nonoclonal antibody (ED7) directed against CD11B/CD18 integrin, which is expressed by macrophages, was used to investigate the pathogenetic effects of macrophages in Adriamycin nephropathy. Male Wistar rats were treated with ED7 antibody, one day prior to Adriamycin (ADR, 7.5 mg/kg) treatment, or 7 days post ADR when overt proteinuria was established. 6 rats each from 4 groups (A. ADR alone, B. ED7 post-ADR, C. ED7 pre-ADR and D. normal control) were sacrified at week 4. Renal function, morphological features and infiltrating cells were assessed. Creatinine clearance was significantly ameliorated by ED7 given pre-ADR (C vs A, $p<0.01$), but not when given post-ADR (V vs A, p=NS). However, proteinuria was not alleviated by either ED7 treatments. Morphometric analysis showed less FSGS with ED7 given pre-ADR compared to ADR alone (C vs A, $p<0.01$), but not when given post-ADR (B vs A, p=NS). Tubular atrophy was reduced by ED7 given pre-ADR (tubular cell heights and tubular diameter (C vs A, $p<0.01$ and $p<0.001$ respectively), and less interstitial expansion (C vs A, $p<0.01$). Tubular atrophy and interstitial expansion with ADr were unaffected by ED7 given after ADR. Cortical macrophage infiltration was reduced by 50% compared to ADR alone by ED7 given before or after ADR. The number of cortical CD4+ T cells appeared to decline with ED7 given pre-ADR, but not with ED7 treatment after ADR. ED7 given pre-ADR, but not post-ADR, protected both renal function and structure in this model of chronice proteinuric renal disease. This suggests that macrophages play an important role in the development rather than the progression of Adriamycin nephropathy.

P86

BLOCKADE OF CD40-CD50 LIGAND REDUCED RENAL INJURY IN CHRONIC PROTEINURIC RENAL DISEASE

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In murine lupus nephritis, blockade of CD40-CD40L pathway inhibits CD40 mediated B-cell activity, reducing severity of the renal lesion. We hypothesised that CD40-CD40L could contributre to renal injury in non-autoimmune diseases as well, and that an anti-CD40L antibody (Mrl) could diminish inflammation and fibrosis in murine Adriamycin nephropathy (ADR). Male BALB/c mice were divided into three groups (n=6 in each group): A. ADR+Mrl, B. ADR only and C. saline-treated control. In group A, mice were treated with intraperitoneal injections of Mrl (0.4 mg/per mouse) at day 5, 7 and 9 after ADR treatment. Mice were sacrified at week 6. Changes in renal function and histopathological features were assessed. Mice treated with ADR and Mrl had significanly less glomerular injury than ADR alone [glomerular surface area (μm^2): A 3142±675 vs B 2463±630, $p<0.001$ and vs C 3292±569, p=NS; glomerular sclerosis (%): A 21.1+6.9 vs B 30.2±10/2, $p<0.001$]. CD40L blockade significantly reduced tubulonterstitial injury as well [tubular diameter (μm): A 42.5±6.9 vs B66.3±13.7, $p<0.001$ and vs C 37.3±5.7, $p<0.02$; tubular cell heights (μm); A 16.3±1.7 vs B 11.0±1.8, $p<0.01$ and vs C 18.2±1.9, $p<0.05$; interstitial volume (%): A 13.9±5.1 vs B 26.2±6.9, $p<0.001$ and vs C 1.3±0.7, $p<0.001$]. Proteinuria (mg/12 hr) in group A was significantly ameliorated (1.8±0.6 vs B 4.3±0.8, $p<0.001$, and vs C 0.7±0.2, $p<0.01$). Creatinine clearance (ul/min) was improved by Mr1 treatment (A 75±4 vs B 35±15, $p<0.001$ and vs C 82±4, $p<0.01$). Blockade of Cd40-CD40L interaction protects against renal structural and functional injury in this murine model of chronic proteinuric renal disease. The mechanisms by which CD40-CD40L interation causes renal injury, and by which Mr1 is protective, are under invesitagation.

P87

RECONSTITUTION OF CD4+ T CELLS REDUCES RENAL INJURY IN SCID MICE WITH ADRIAMYCIN NEPHROPATHY

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CD4+ T cells are major infiltrating lymphocytes in chroic tubulointerstial inflammation associated with nonimmunological renal disease. In this study, mice with Adriamycin nephropathy was produced in mice deficient in T cells and B cells (SCID mice) and reconstituted with CD4+ T cells or leukocytes without CD4+ T cells, to investigate the role of CD4+ T cells in the progression of Adriamycin nephropathy (ADR). SCID mice were divided into 4 groups: A. ADR only, B. ADR reconstituted with CD4+ T cells, C. ADR reconstituted with leukocytes without CD4+ (non-CD4+ leukocytes) and D. normal control. SCID mice developed proteinuria with Adriamycin (5 mg/kg) and were reconstituted with CD4+ T cells (3×10^6 per mouse) or the same number of leukocytes with CD4+ T cells at week 2 and week 4 after Adriamycin. Renal function and morphological features were assessed at week 6. Glomerular sclerosis was significantly reduced by CD4+ reconstitution ($p<0.001$), but increased by reconstitution with non-CD4+ leukocytes ($p<0.01$) in comparison to unreconstituted SCID-ADR mice. Similarly, reconstitution with CD4 T cells resulted in less tubule injury (tubular diameter and tubular cell height: B vs A, both $p<0.01$, and B vs C, $p<0.001$ and $P<0.005$ respectively]. Interstitial volume was significantly reduced in mice reconstituted with CD4+ ($p<0.01$), but increased in mice reconstituted with non-CD4+ leukocytes ($p<0.001$). The ratio of urine protein/urine creatinine in mice with CD4+ reconstitution was reduced significantly (B vs A, $p<0.01$ and vs C, $P<0.001$). Serum creatinine was not significantly different between ADR+CD4+ group and ADR alone, but increase increased in ADR+non-CD4+ leukocytes ($p<0.01$). Reconstitution with CD4+ T cells protects against progression of renal injury. However, reconstitution with non-CD4 leukocyts (CD8, NK cells and monocytes) accelerates nephropathy in this model of chronic proteinuric renal disease.

P88

ANTI-CD3 MAB TREATMENT BLOCKS HEYMANN NEPHRITIS BY INHIBITION OF $CD8^+$ CELLS AND AN ENHANCED TH2 RESPONSE

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Heymann Nephritis (HN) is a rat model of human membranous nephritis in which auto-antibody (auto-Ab) and complement have been thought to be the mediators of injury. Recently $CD8^+$ cytotoxic Th1 cells have been implicated as effectors of glomerular injury. This study further investigated the role of $CD8^+$ Th1 cells in active HN by treatment with an anti-CD3 monoclonal-Ab (mAb; G4.18) that can block Th1 and cytotoxic T cell function. Anti-CD3 mAb therapy from the time of immunization of renal tubular antigen (RTA) (0–5 weeks) or pre-proteinuria (4–6 weeks) prevented proteinuria but late treatment (7–12 weeks) post-proteinuria had no effect. Compared to HN positive controls early Anti-CD3 mAb treatment delayed anti-RTA responses, reduced $CD4^+$ and $CD8^+$ T cells in peripheral blood and the lymph node (LN) draining the site of immunization, and increased expression of the Th2 cytokines of IL-4 and IL-5 mRNA in nodes. Late treatment had no effect on anti-RTA antibodies. Glomerular deposition of IgG and complement (C3 and C9) was not effected by anti-CD3 mAb therapy. In the glomeruli and renal cortex, cellular infiltration of TCR-$\alpha\beta^+$ cells, $CD8^+$ cells and macrophages in anti-CD3 therapy with reduced proteinuria were significantly less than HN controls. This study demonstrated that anti-CD3 mAb therapy prior to the development of proteinuria blocked infiltration of $CD8^+$ cells and macrophages and prevented proteinuria without effecting glomerular Ig and C deposition.

P89

IS MYCOPHENOLATE MOFETIL (MMF) LESS SAFE THAN AZATHIOPRINE IN OLDER RENAL TRANSPLANT PATIENTS?

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MMF has been shown to be superior to Azathioprine (Aza) in preventing early acute rejection in the general renal transplant population. However, it is uncertain whether these benefits also apply to older renal transplant recipients, who are known to be more susceptible to infections and have lower rates of rejection. To address this issue, a retrospective analysis was undertaken of all elderly (≥55 years-old) renal transplant recipients who underwent renal transplantation at our centre (1994–2000) and received either MMF (n=60) or Aza (n=55) in combination with prednisolone and cyclosporin. The two groups were well matched at baseline. Compared with the MMF cohort, Aza-treated patients experienced a shorter time to first rejection (hazard ratio [HR] 4.47, 95% CI 1.53–13.1, $p<0.01$). However, Aza-treated patients also experienced fewer gastrointestinal complications (4% versus 20%, $p<0.05$) and opportunistic infections (HR 0.11, 95% CI 0.03–0.41, $p=0.001$). No differences were observed in the frequencies of hospitalisation, intensive care admission, haematologic complications or malignancy. Actuarial 2-year survival rates for the Aza- and MMF-treated patients were 100% and 87%, respectively ($p<0.001$). The principal cause of death in the MMF cohort was infection. Using a multivariate Cox regression analysis of patient survival, an adjusted hazard ratio of 0.01 (95% CI 0.001–0.08, $p=0.001$) was calculated in favour of Aza. Immunologic graft survival was not significantly different between the two groups. In conclusion, the combination of MMF, cyclosporin and prednisolone in elderly renal transplant recipients appears to result in a significantly increased risk of gastrointestinal complications, serious opportunistic infections and death compared with the less potent combination of Aza, cyclosporin and prednisolone.

P90

WITH THE BEST OF INTENTIONS: IS IT POSSIBLE TO MEET THE CARI GUIDELINES?

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The CARI[1] draft dialysis guidelines propose evidence based targets for biochemical and haematological parameters in ESRF. As part of a prospective randomised trial we investigated our ability to apply the CARI and National Heart Foundation of Australia targets to a representative dialysis population. All patients aged between 18–80 yrs were encouraged to enroll regardless of prior history of non-compliance or co-morbidity. Patients were randomised to either usual care (U;n=44) or focussed care (F;n=45). Usual care involved monthly blood tests and pysician review second monthly. In addition focus care patients had a monthly review in a physician supervised trial clinic run by nurses. The groups were comparable at baseline in terms of age, gender, dialysis modality, proportion of diabetics, time on dialysis, haemoglobin, ferritin, % saturation, parathyroid hormone, serum corrected calcium, serum phosphate, total cholesterol and LDL. At 6 months there had been significant improvements in PTH ($p<0.05$), total cholesterol ($p<0.05$) and LDL ($p<0.001$), and a trend to better BP control. The proportion of patients meeting targets at 6 months were as follows: tot chol<5 mmol/l-U 63%, F 82%; LDL<3 mmol/l-U 75%, F 91%; phosphate<1.8 mmol/l-U 42%, F 62%; PTH<21 pmol/l-U 21%, F 40%; BP sys<140 mmHg-U 41% F 46%; Hb>11.5 g/dl U 58% F 64%. In spite of an intensive programme to maximise management of the haematological and biochemical parameters in patients with ESRF it appears that in a significant proportion of patients these targets could not be reached.

[1]The CARI Guidelines (Caring for Australians with Renal Impairment). Australian Kidney Foundation & Australia New Zealand Society of Nephrology, 2001.

P91

COMPLIANCE WITH CARI (CARING FOR AUSTRALIANS WITH RENAL IMPAIRMENT) GUIDELINES IN LONG TERM DIALYSIS PATIENTS AT AN IN-CENTRE OR SATELLITE UNIT

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The recent formulation of the CARI guidelines has prompted our review of the quality control of biochemical parameters in the dialysis population. We reviewed several biochemical parameters of our patients (n=119) (in centre (n=57) or satellite (n=62)) and assessed how many patients' results were in keeping with the current draft CARI guidelines. The biochemical parameters assessed were albumin corrected serum calcium (s.Ca) and phosphate (s.PO4), parathyroid hormone (PTH) and the calcium phosphate (CaPO4) product was calculated. The table below demonstrates the percentage of in-centre and satellite patients whose biochemical parameters were in accordance with the CARI guidelines. Of concern was the 20% (24/119) of patients who had s.PO4 levels that have been independently associated with increased

Parameter	Guideline	In-Centre (%) Compliance	Satellite (%) Compliance
s.Ca	2.15–2.55 mmol/L	64.5%	66.6%
s.P04	<1.8 mmol/L	51%	66%
CaP04	<4.5	47%	63%
PTH	12–18 pmol/L	16%	13%

mortality. Also, CaPO4 product was not routinely monitored. Only 6.7% (8/119) patients were able to comply with the CARI guidelines in all four areas. We also review these results in the context of their current prescribed medications and compare guideline compliance between the in-centre and satellite patients. These results represent less than ideal control of several biochemical parameters. Although patient compliance hinders guideline fulfilment the role medications and physicians play needs to be assessed.

P92

WELLINGTON RENAL DEPARTMENT ANAEMIA SURVEY

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Anaemia management in the dialysis population has improved with the use of erythropoietin (Eprex, Jannsen-Cilag) and a greater understanding of iron requirements. This study is an audit of the Wellington Renal Department's dialysis population (n=178, haemodialysis (HD)=95, peritoneal dialysis (PD)=83) aiming to assess the haemoglobin (Hb) concentrations (current aim 110–130 g/L), use of Eprex and identify contributing factors. Tests are t-tests for comparisons and Spearman rank correlations. The mean Hb (±95% CI) was 109±3 g/L with no difference between HD and PD. Compared to all other patients a lower Hb was noted at the satellite incentre HD unit in Hastings, mean Hb=99±8 g/L, $p=0.037$. 44.3% of patients were receiving Eprex (mean dose=6468±693 IU/week). The Hb was no different in those receiving Eprex and those not, (107±3 v 110±4 g/L). Serum ferritin was no different among the various dialysis subpopulations (mean=341±65 ng/ml). Transferrin saturation (TSAT) was also similar across the group (mean=25±2%). A negative correlation was seen between ferritin and Hb in those not receiving Eprex ($r=-0.42$, $p<0.0001$), although not seen in those receiving Eprex ($r=-0.11$, $p=0.35$). Of those who were anaemic (Hb<110 g/L) or on Eprex, 28 had absolute iron deficiency (ferritin<100 ng/ml) and 25 had relative iron deficiency (ferritin>100 ng/ml, TSAT<20%). A negative correlation was noted between Eprex dose and TSAT ($r=-0.25$, $p=0.03$) but not ferritin ($r=-0.12$, $p=0.29$). A correlation was seen between serum albumin and Hb for those patients not on Eprex ($r=0.31$, $p=0.0016$) but not for those on Eprex. No correlations were seen between Hb± Eprex with TSAT, red blood cell folate, vitamin B12, parathyroid hormone, aluminium, age, urea reduction ratio (HD) or weekly residual and total creatinine clearances (PD). Only 2 further patients were identified that qualified for a subsidised Eprex prescription. In conclusion, we are not achieving our goal Hb despite use of Eprex with iron deficiency a common contributing factor.

P93

A 12-MONTH REVIEW OF CAPD PERITONITIS IN WA: HOW APPLICABLE ARE THE ISPD GUIDELINES?

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Background The ISPD guidelines advocate against the empirical use of vancomycin and gentamicin for CAPD peritonitis. The Western Australia CAPD fluid cultures over 12 months were reviewed to determine the feasability of following these guidelines statewide.

Method We retrospectively obtained peritoneal fluid culture results from the 3 major teaching hospitals (SCGH, RPH and FH) servicing the whole adult population of dialysis patients in WA from 31/1/00 to 31/1/01. These institutions serve different geographical areas with different racial demographics. The data was analysed by dividing the isolates into methicillin-resistant Gram-positive bacteria (MRGPB), multi-drug resistant Gram-negative bacteria (MDR-GNB, resistant to 3rd generation cephalosporins/ciprofloxacin) and fungi versus susceptible organisms. Variables compared with antimicrobial resistance profiles were institution, aboriginality and demographics (metropolitan vs regional). The data was obtained through hospital databases and dialysis records.

Results Of the 286 patients studied, 30% were aboriginal and 34% were regional patients. The peritonitis rate was 0.51 per patient per year overall and highest (0.88) in regional aborigines.134 CAPD fluid specimens from episodes of peritonitis with susceptibilities were identified (133 patients, 57 specimens from SCGH; 129 patients, 66 specimens RPH; and 24 patients, 11 specimens FH). 21.6% of the total specimens contained MRGPB (15.7% Staphylococcus epidermidis, 3% MRSA), 1.5% MDR-GNB and 6% of cultures yielded fungi. No MRGPB were resistant to vancomycin and no MDR-GNB were resistant to gentamicin. 20% of cultures in non aboriginal patients yielded MRGPB compared with 17.2% in aboriginal patients ($p = NS$). There was no statistical significant difference in resistant isolates between the 3 hospitals. 12 episodes of clinical peritonitis from the regional patients of RPH were not available for analysis. Aboriginal patients had 6 of the 7 cases of fungal peritonitis ($p < 0.05$).

Conclusions. There were no significant differences in antimicrobial resistance patterns between aboriginal and non-aboriginal patients and institutions. The ISPD guidelines can be safely applied to 70.9% of peritonitis episodes in Western Australia except in the cases of known carriers of MRSA or MRGPB. Empirical therapy with gentamicin can be avoided given the low rates of MDR-GNB.

P94

RAPID ASSESSMENT OF URINARY PROTEIN EXCRETION IN RENAL DISEASE

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BACKGROUND: 24 hour urine collection is the gold standard for assessing urinary protein excretion but is inconvenient and prone to collection errors. Spot urine protein to creatinine ratio (P/Cr) has been proposed as an alternative measure, but only 6 studies have examined the relationship between 24 hour urine protein excretion and P/Cr. None of these have provided sufficient regression analysis to allow reliable extrapolation of spot P/Cr to 24 hr protein excretion.
AIM: To determine whether a reproducible relationship exists between random spot urine P/Cr measured the day of clinic visit and subsequent 24 hour protein excretion collected on a different day, as occurs in usual clinical practice.
METHODS: Patients with renal and/or hypertensive disorders had a spot urine sample tested by automated dipstick (Bayer Clinitek 50) followed by autoanalyser measurement of protein (benzethonium method) and creatinine concentrations and spot P/Cr calculated. The same patients underwent 24 hr urine collection for the same measures a median of 5 (range 0–93) days later. Patients' age, weight, renal diagnosis, serum creatinine, blood pressure and urinalysis were recorded.
RESULTS: To date 39 patients have complete data, with an age of 56 (18) yrs, 43% male. Spot urine P/Cr correlated significantly with subsequent 24 hr urine protein excretion ($r = 0.78$, $p < 0.0001$) and the regression equation describing this relationship was: 24 hr protein excretion (g/d) = 0.17 + 8.5 spot P/Cr (g/mmol).

There was a significant but weak correlation between dipstick proteinuria and measured urinary protein concentration ($r = 0.59$, $p < 0.0001$) and between dipstick proteinuria and both the spot P/Cr ($r = 0.51$, $p < 0.0001$) and the subsequent 24 hour protein excretion ($r = 0.51$, $p < 0.0001$).

CONCLUSION: Preliminary indications from this study are that a significant relationship exists between the spot P/Cr tested on the day of assessing the patient and 24 hour urine protein excretion measured on a different day. If substantiated when a larger number of patients are recruited, this would permit use of spot P/Cr routinely in clinical practice.

P95

ACCURACY OF DIPSTICK TESTING FOR POPULATION SCREENING

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Screening for early renal disease may provide an opportunity to halt the ever-increasing burden of end-stage renal failure. Dipstick testing of urine for proteinuria, haematuria and urinary tract infection is cheap, accessible and has acceptable sensitivity and specificity when used in a clinical setting. The lower prevalence of disease in a community setting has implications for the positive (PPV) and negative (NPV) predictive values. AIM. To assess the accuracy of dipstick testing in a community setting. METHODS. 200 consecutive adults aged ≥ 25 years residing in Tasmania were asked to provide a mid-stream urine sample. 3 aliquots were tested: (1) dipstick for blood, protein, nitrites, leukocyte esterase (1+ or greater = positive); (2) protein : creatinine ratio (ratio > 20 mg/mg abnormal); (3) culture and microscopy. RESULTS: 297 subjects completed testing:

Indicator	Sensitivity	Specificity	PPV	NPV
Haematuria	29.6%	97.5%	85.3%	73.8%
Proteinuria	83.3%	86.6%	11.4%	99.6%
Leuco. Est.	69.2%	85.9%	18.4%	98.4%
Nitrite	30.8%	99.3%	66.6%	96.9%
Leuk +/– But	69.2%	85.6%	18.0%	98.4%

CONCLUSIONS. Dipstick testing performed on a population basis for proteinuria and haematuria provides an acceptable PPV and a high NPV rendering it suitable for screening for early renal disease. Screening may be a realistic means by which to reduce the burden of ESRD.

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NOVEL ERYTHROPOIESIS STIMULATING PROTEIN (DARBEPOETIN ALFA) ADMINISTERED ONCE EVERY OTHER WEEK CORRECTS ANAEMIA IN PATIENTS WITH CKD

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Darbepoetin alfa is a novel erythropoiesis stimulating protein with an approximately 3-fold longer terminal half-life than rHuEPO. The purpose of this study was to evaluate the effectiveness of fixed doses of darbepoetin alfa administered SC once every other week for the treatment of anaemia in patients with chronic kidney disease (CKD).

Eligible patients had a mean baseline haemoglobin (Hb) value < 11.0 g/dL, adequate iron stores (TSAT > 20% or serum ferritin > 100 ng/mL), creatinine clearance < 30 mL/min and had not received rHuEPO (rHuEPO-naive) within the previous 12 weeks. The starting dose of darbepoetin alfa was 0.75 mcg/kg rounded to the nearest fixed dose (i.e., 10, 15, 20, 30, 40, 50, 60, 80, 100, 130, and 150 mcg). Dose was titrated, as necessary, to achieve and maintain target Hb (11.0–13.0 g/dL).

Analysis of the first 23 enrolled patients to complete at least 10 weeks of darbepoetin alfa treatment showed that the mean (SD) baseline Hb was 9.83 + 0.73 g/dL and the mean increase in Hb over the initial 4 weeks of darbepoetin alfa treatment was 1.37 + 0.81 g/dL. The median time to achieve a Hb response (2 consecutive Hb measurements > 11.0 g/dL) was 6 weeks (range: 0–17 weeks) and 95% of patients reached target Hb range within 10 weeks of initiating darbepoetin alfa therapy (95% CI: 73.2%, 97.6%). At the time of Hb response, the median darbepoetin alfa dose was 50 mcg every other week (range: 30–130 mcg). At least one dose adjustment prior to achieving the Hb target range was required by 43% of patients. The safety profile of darbepoetin alfa was consistent with that expected for CKD patients.

In conclusion, darbepoetin alfa administered as fixed doses once every other week is effective for the treatment of anaemia in rHuEPO-naive patients with CKD.

Key words: anaemia; erythropoiesis; chronic kidney disease

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ONE YEAR FOLLOWED-UP OF TREATMENT OF PRIMARY GLOMERULONEPHRITIS IN DR. CIPTO MANGUNKUSUMO HOSPITAL, JAKARTA

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The aim of this study was to evaluate the remission and relaps rate of 13 patients with primary GN based on the renal biopsy. Only 13 of 59 patients who had renal biopsy in the year of 2000 from Nephrology Division of Dr. Cipto Mangunkusumo General Hospital could be assessed completely. Minimal Change Disease (MCD) was found in 9 patients, mesangial proliferative GN in 2 patients, focal segmental GN and IgA nephropathy in 1 patient consecutively. Prednison 1 mg/kg/body weight was given for 6–8 weeks, and then was tapering to 10 mg/weeks. During 3 months followed-up 8 out of 9 MCD patients had completely remission characterized by no clinical symptoms without proteinuria while 1 patient had spontaneous remission. Two out of 5 patients with non MCD had completely remission, 2 patients had partial remission characterized by no clinical symptoms with proteinuria, and 1 patient failed to response in the treatment scheme ($p = 0.02602$). During 6 months followed-up 8 MCD patients had complete remission. In the non MCD group, 1 had complete remission, 2 patients had partial remission, and no response in 1 patient ($p = 0.007$). After 9 months followed-up 5 out of 8 MCD patients had complete remission and 1 patient was found relaps characterized by proteinuria after remission period. In non MCD group, 1 patient had complete remission, 2 patients had partial remission, and 1 patient showed no response ($p = 0.82701$). During the 12 months followed-up 5 out of 8 MCD patients had complete remission and 1 patient was found relaps while in non MCD group, only 1 patient had complete remission, 1 patient had partial remission and 1 patient showed no response ($p = 0.85098$). Conclusion : MCD group showed completely remission during 3 and 6 months followed-up; while after 9 and 12 months no differences was found.

P98

DO PLASMA NON-ESTERIFIED FATTY ACIDS AND INSULIN RESISTANCE CONTRIBUTE TO IMPAIRED ENDOTHELIAL FUNCTION IN NEPHROTIC SYNDROME?

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OBJECTIVE: To assess the role of insulin resistance and plasma non-esterified fatty acids (NEFA?s) in endothelial dysfunction as measured by brachial flow-mediated dilatation (FMD) in nephrotic syndrome (NP).
METHODS: FMD was compared between NP ($n = 21$) and controls, NC ($n = 20$). Plasma glucose, insulin and NEFA?s were measured and body mass index (BMI, kg/m2), waist-hip ratio (WHR) and insulin resistance were calculated using the HOMA score (homeostasis model assessment).
RESULTS: FMD was significantly lower in the NP group (NP $4.9\% \pm 0.6$, NC 8.0 ± 0.5, $p < 0.001$). There was a trend to higher WHR in the NP group (NP 0.88 ± 0.02, NC 0.82 ± 0.02, $p = 0.06$). Fasting insulin (NP 12.8 ± 1.4, NC 6.8 ± 0.7 mU/L, $p = 0.001$) and HOMA (NP 3.0 ± 0.4, NC 1.5 ± 0.2, $p = 0.001$) were significantly higher in NP, with a trend to a higher glucose (NP 5.3 ± 0.2, NC 4.9 ± 0.1, $p = 0.054$). These differences persisted after adjusting for WHR. There was a trend to lower plasma NEFA in the NP group (NP 0.27 ± 0.02, NC 0.37 ± 0.04, $p = 0.052$). NEFA levels correlated positively with serum albumin ($r = 0.39$, $p = 0.023$), but not with HOMA, WHR, or serum triglyceride. In the NP group, regression analysis showed that FMD was negatively correlated with NEFA independent of HOMA, LDL-cholesterol, blood pressure and serum albumin ($b = -0.58$, $p = 0.021$).
CONCLUSION: Endothelial function in NP is inversely correlated with NEFA but not insulin resistance. We postulate that in NP, low serum albumin may facilitate increased binding of NEFA?s to the endothelium where they may contribute to impaired nitric oxide synthase activity and to lipoproteins where they can increase atherogenicity.

P99

COMPARISON OF THE EFFECT OF 50% INCREASE IN ANGIOTENSIN CONVERTING ENZYME INHIBITOR (ACEI) WITH A COMBINATION OF CANDESARTAN AND ACEI ON PROTEINURIA AND BLOOD PRESSURE IN CHRONIC RENAL DISEASE

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We have reported a randomised controlled trial in 60 patients in which addition of Candesartan to ACEI treatment significantly reduced proteinuria (JASN 2000, 11, 1349A). In the final period of this trial Candesartan was ceased and the dose of ACEI was increased by 50%. The effect of this 50% increase in ACEI on proteinuria, blood pressure and renal function was compared with the ACEI plus Candesartan period. Urine protein was 1.83 grams/24 hours on Candesartan & ACEI and 2.4 grams/24 hours when ACEI dose was increased by 50% (Difference 0.57 grams/24 hours 95% CI 0.1–1.0 $p < 0.015$). This significant rise in proteinuria when Candesartan was ceased and ACEI dose was increased by 50% was accompanied by a rise in systolic blood pressure (SBP). SBP was 126.79 on ACEI and Candesartan and 134.47 on ACEI increased by 50% (difference 7.66 CI 3.1–12.2 $p < 0.002$). Diastolic blood pressure, serum creatinine, urea and potassium were unchanged. Conclusion: When an ARA is combined with an ACEI the urine protein level and systolic blood pressure are lower than when ACEI dose is increased by 50%.

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CONTINUING FALL IN PROTEINURIA AFTER 6–9 MONTHS OF COMBINED ANGIOTENSIN CONVERTING ENZYME INHIBITOR (ACEI) AND ANGIOTENSIN II RECEPTOR ANTAGONIST (ARA) WITH DISAPPEARANCE OF PROTEINURIA OF 6–21 YEARS DURATION IN 5 PATIENTS

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At the 2000 ASN meeting we reported a randomised controlled trial comparing ACEI with combined ACEI and ARA in chronic renal disease with stable proteinuria (JASN 2000, 11, A1825). ACEI combined with ARA lowered urine protein and blood pressure more than ACEI alone. After trial completion participants were offered continuing ACEI and ARA treatment and 31 patients with documented proteinuria over 3–27 years and now have been followed for at least 6 months after trial completion on combined ACEI and ARA. In 21 of the 31, urine protein levels are lower at 6–9 months than in the 3 month ACEI and ARA period of the trial. In the group of 31 reduction in urine protein was significant. Mean difference in urine protein comparing 3 months with 6–9 months of ACEI and ARA 0.4 grams ($p < 0.02$ 95% CI 0.5–0.75). In 5 patients with proteinuria of 6–21 years duration and maximum proteinuria levels of 1.7–8.2 grams, urine protein levels fell for the first time to the normal range (<0.16 grams/24 hours) on combined ACEI and ARA over 6–9 months. Diagnoses were in these 5 were membranous glomerulonephritis (2), mesangiocapillary glomerulonephritis Type 1 (1), reflux nephropathy (1), Henoch-Schönlein nephritis (1). Conclusion: Combining ARA with ACEI appears to have a continuing effect on proteinuria beyond 3 months. Longer follow-up studies are indicated.

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SILDENAFIL AND ERECTILE DYSFUNCTION IN MALE RENAL PATIENTS

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Erectile dysfunction (ED) is a common and distressing problem. Sildenafil is a potent inhibitor of cyclic guanosine monophosphatase specific phosphodiesterase enzyme in the corpus cavernosum. This medication has been shown to be effective in the treatment of ED, however it's use in renal disease is limited. We report our experience with sildenafil.

Sixteen patients were seen at a dedicated renal impotence clinic. Four patients had chronic renal failure independent of dialysis, four were on peritoneal dialysis, three were on haemodialysis and five had functioning renal transplants. The duration of ED ranged from 12 to 78 months. Six patients were unable to form erections.

Initial evaluation was made to optimise the patients general condition, substitute implicated medications, correct anaemia, and evaluate and treat sex hormone and thyroid abnormalities prior to use of sildenafil.

Following treatment 15 out of 16 patients were able to achieve an erection, with fourteen able to achieve satisfactory intercourse. The dose required was 25 mg for 3 patients, 50 mg for 4 patients and 100 mg for 7 patients. No change in libido was noted. 6 patients reported an improvement in their relationship with their partner, and ten an improvement in self-esteem.

Seven patients suffered side-effects, however all were able to continue treatment. Two patients had mild flushing, two patients had headache, one patient gastro-oesophageal reflux, one patient priapism and one patient palpitations.

Initial experience suggests sildenafil is a safe and effective medication in the treatment of ED for male renal patients.

P102

A CHRONIC DISEASE OUTREACH PROGRAM FOR REMOTE ABORIGINAL COMMUNITIES: EXPERIENCE AT ONE YEAR

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The Tiwi Treatment Program has shown that adequate diagnosis and treatment of chronic disease (hypertension, albuminuria and diabetes), greatly reduces morbidity, renal failure, and premature deaths, and saves costs from dialysis avoided. Some regional health services have been slow to implement these principles, so we designed a program to help improve awareness and management of chronic disease in other Aboriginal communities in remote areas. The program espouses that chronic disease surveillance be incorporated into regular adult health care. Its operational focus is education, training and empowerment of Aboriginal health workers (AHWs) to run the program. It relies on algorithms for testing & treatment, with professional backup as needed. Minimal testing includes weight, skin exam, BP, random glucose & urinary dipstick, with other tests as needed. It uses a web-based interactive database, for assessment and input from afar and ongoing evaluation. Community health profiles are generated as work proceeds. Participation is by community request. Three Top End NT communities are participating- Borroloola in the Western Gulf, (population 1350), Nauiyu Nauiyu (pop 470) and Wadeye (pop 2050). Challenges include costs, duration, discomforts and hazards of trips by small plane or 4WD; seasonal inaccessibility due to floods, scarce accommodation, limited literacy in helpers, lack of facilities and clinical equipment, set-up and service of computers, and poor condition and transmission capacity of phone lines, impeding data transfer. Although the primary responsibility for assessments and management is with health workers and nurses, a doctor must be willing to confer regularly, even from far, and endorse treatment plans. AHWs have quickly grasped the concepts and skills, and have presented at workshops and national meetings. 180 people have been tested at Naiuyu, (start date May 2000), where BPs have already improved, and 120 have been tested in each of the other communities (started Nov 2000). Health profiles are substantially different in the three communities. Contributions from the Colonial Foundation and Janssen Cilag will allow expansion to other regions in 2001.

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HELICOBACTER PYLORI (HP) INFECTION, CHRONIC RENAL DISEASE AND RENAL DEATH

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Australian Aborigines have high rates of renal disease, which is multideterminant. Birth weight, body habitus, metabolic and hemodynamic profiles, heavy drinking and skin infections influence risk. We now describe its association with antibodies to HP.

509 people in one high risk community (228 females, 281 males, age 14 to 76 yr), had a health screen between 1992 and 1996. Tests included the urine albumin/creatinine ratio (ACR, gm/mol), and HP-antibody assays by microimmunofluorescence (Pyloriset EIA-G 111). They were followed until start of dialysis, death or until June 30, 2000, and survivals calculated by Cox proportional hazard method.

Mean follow up was 6.1 yr (SD 1.8). There were 54 natural deaths, of which 19 were renal deaths (dialysis or death with chronic renal failure). HP titers at baseline exam ranged from 45 to 8000; overall 33.4% were "seropositive" (titre ≥ 300), with no change with age. Females were more often HPpos than males, 38.5% vs 29,1%, p = 0.025. The table shows that HPpos people had higher ACRs and creatinines at baseline, and higher rates of renal death and all-cause natural death.

	HPneg	HPpos	P
ACR, gm/mol, g mean (CI)	3.0 (2.4–3.8)	6.1 (4.4–8.5)	0.001
ACR 34+ (OR, CI)	20.0%	32.4%	0.002
Serum creatinine, umol/L	83.1 (2.2)	93.6 (3.1)	0.026
Renal death, rate/100 person yr	0.3 (0.2–0.7)	1.1 (0.6–1.9)	0.017
Natural death, rate/100 person yr	1.3 (0.9–1.9)	2.6 (1.8–3.7)	0.013

The adjusted hazard ratio (CI) of HPpos vs HPneg people for renal death was 2.88 (1.2–7.4) and for all-cause natural death was 1.87 (1.1–3.3). Significance persisted after accounting for diabetes, blood pressure, lipids and baseline ACR. Antibodies to Chlamydia Pneumoniae, whoever, did not correlate with renal disease or deaths.

Serologic evidence of HP infection correlates with renal disease in this high risk environment, and predicts renal deaths and all cause deaths. The mechanism might be causal, or HP antibodies might mark the relative burden of illness that influences health and mortality in this and other disadvantaged Aboriginal groups.

P104

GLOMERULAR NUMBER, GLOMERULAR VOLUME AND KIDNEY WEIGHT IN FORENSIC AUTOPSIES: A MULTIRACIAL STUDY

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We report glomerular number (Nglom), glomerular corpuscle volume (Vcorp), and kidney weight (kidwt) in a forensic autopsy study of people with no a priori suspicion of renal disease. There were 63 subjects: 25 US Blacks, 16 US whites, 9 Australian Aborigines and 13 Australian whites; 47 males and 16 females, newborn to 84 years, with 10 people < 18 yr old. The right kidney was perfusion-fixed and sub-sampled for stereological estimation of Nglom and mean Vcorp using the physical dissector/fractionator combination. Nglom and Vcorp were normally distributed. Nglom ranged from 210 332 to 1 825 380, an 8.7-fold difference, with a mean (SD) of 747 044 (328 804). Nglom fell after age 50. Females had 19% fewer glomeruli than males. Vcorp increased dramatically during childhood, and ranged from 3.51 to 19.55 $\mu m^3 \times 10^6$ in adults, a 5.6-fold difference, with a mean (SD) of 9.79 (3.52). It did not differ by sex. Nglom was inversely correlated with Vcorp in adults (p = 0.003). There were no significant differences in Nglom or Vcorp, and therefore in total glomerular volume, by racial group. However, kidwt was lower in Blacks than Whites, 165.4 (9) vs 210.7 (13) gm, p = 0.008, adjusted for sex and age. This was associated with reduced extraglomerular mass (EGM), which was due to smaller increments of EGM with increasing BSA in Blacks than Whites—regression predicted an increase in EGM per m^2 increase in BSA of 37.6 (CI 15,91) gm, (p = 0.145) in Blacks and 104.8 (35 174) gm, (p < 0.005) in Whites.

The range of Nglom is much greater than the 3.7-fold range reported by Nyengaard and Bendtsen (Anat Rec 232:194–201, 1992). The wide range of Vcorp has not been described before. The inverse relationship between Vcorp and Nglom suggests that glomeruli hypertrophy in response to reduced nephron number. Hyperperfusion of these larger glomeruli probably predisposes to glomerulosclerosis. Racial differences in Nglom and Vcorp are not yet apparent, but among Blacks, there appears to be a reduced capacity to increase extraglomerular structures with enlarging body size compared to whites.

P105

TIMING OF NEPHROLOGY REFERRAL: ITS PREDICTORS AND INFLUENCE ON PATIENT OUTCOME

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We retrospectively studied the influence of late referral (< 16 weeks pre-dialysis) on the morbidity and mortality of all patients attending the Statewide Renal Service who started haemodialysis between January 1999 and December 2000 (n = 228) and factors associated with late referral. Patient outcome differed significantly according to referral pattern (Table 1).

Variable	Early referral N = 100	Late referral N = 128	P value Chi^2 test
Death	14 (14%)	49 (38%)	< 0.001
Hospitalisation	22 (22%)	101 (79%)	< 0.001
Vascular access infection	6 (6%)	24 (19%)	0.003

Late referral was significantly associated with living in rural New South Wales (n = 46, p = 0.005), being non-Caucasian (n = 99 p < 0.001), being privately insured (n = 57, p = 0.025), and presenting directly to the emergency department (n = 48, p = 0.005). There were no significant differences between the two groups according to age, sex or body mass index (BMI). In logistic regression models late referral was predicted independently by non-Caucasian racial origin (OR 7.69, CL 1.35, 50.00), direct presentation (OR 3.22, CL 1.45, 7.14) and private health insurance (OR 2.27, CL 1.19, 4.35). Mortality was significantly higher in patients who were Indigenous Australians (OR 15.24, CL 2.69, 86.44), and referred late to the renal unit (OR 2.70, CL 1.16, 6.25). Differences in patient outcome according to referral pattern suggest that strategies to increase early nephrology referral should be a priority while recognition of factors influencing late referral identifies the possible focus of such strategies.

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ACCELERATED PROGRESSION OF CALCIFIC AORTIC STENOSIS IN DIALYSIS PATIENTS

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Abnormalities of the aortic valve occur with increased frequency in patients with renal failure and may contribute to the excess cardiovascular mortality in this patient group. No controlled data is available regarding the rate of progression of aortic stenosis in dialysis patients as compared to the general population. A retrospective case-control study was thus performed to examine this question. Dialysis patients with aortic stenosis were identified by a search of the echocardiography database. 28 dialysis patients were compared to 56 contemporaneous sex matched controls without renal failure. All had aortic stenosis on at least 2 echocardiograms more than 6 months apart. Changes in mean and peak transvalvular gradient as well as valve area were calculated from echocardiographic data and compared. The initial characteristics of the two patient groups were similar although the dialysis group had a greater median baseline valve area (1.55 vs 1.30 cm^2, p = 0.01) and shorter median follow up (1.6 vs 2.6 years, p = 0.02). Aortic stenosis progressed significantly more rapidly in the dialysis patients than the controls when measured by change in valve area (−0.19 vs −0.07 cm^2/year respectively, p < 0.001) and change in peak transvalvular gradient (6.5 vs 3.9 mmHg/year, p = 0.04). There was also a trend towards more rapid progression of mean transvalvular gradient (4.9 vs 2.5 mmHg/year, p = 0.052). On multivariate linear regression analysis, only end stage renal failure (p = 0.02) and baseline valve area (p = 0.04) predicted accelerated progression of aortic stenosis, while age, sex, baseline transvalvular gradient and length of follow up had no effect. Aortic stenosis progresses more rapidly in dialysis patients. The timeframes for review and operation in dialysis patients should be shorter than for the general population.

P107

RISING END STAGE CHRONIC RENAL FAILURE (ESCRF) RATES IN THE PILBARA REGION OF NORTH-WEST WESTERN AUSTRALIA

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To document the prevalence of renal disease in the Pilbara, define future needs and improve on existing intervention strategies, statistics on renal and diabetes referrals to our medical unit have been kept over a $4^1/_2$ year period. Intervention strategies are outlined and prevalence data from January 1998 is compared with data from June 2001.

532 patients have been followed. Interventions have included establishment of a local haemodialysis centre, introduction of specialist services to 7 remote communities, utilisation of a computerised database, shared care arrangements with primary caregivers emphasising opportunistic screening and standard medical and lifestyle interventions.

Numbers of patients in this period on ESCRF treatment has risen from 17 to 32 all indigenous (3–4000 per million population). Annual ESCRF mortality was 12%. Numbers under aged 60 years with established renal impairment and/or severe proteinuria (albumin/creatinine ratio > 100) rose from 43 to 125. Over 3 years, 38% such high-risk patients (n = 16) progressed to ESCRF treatment and 25% died. There were 21 new acceptances, 25% (n = 5) presented late.

These figures follow national indigenous trends and point to a further doubling of ESCRF patient numbers over the next 3 years unless resources are urgently increased.

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IS MAGNETIC RESONANCE ANGIOGRAPHY (MRA) USEFUL IN RENOVASCULAR DISEASE?

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MRA correlates well with conventional angiography (CA) and enables noninvasive assessment of renal vessels without nephrotoxic contrast. Given the increasing use at our institution, we aimed to identify the referral source and nature of the patient group tested, and the impact of renal MRA on their management. 121 renal MRA scans performed at the Royal Adelaide Hospital from 1.11.97 to 31.12.00 were reviewed. Clinical data was obtained by case note review or from treating physicians. Scans were with gadolinium enhancement, using a Siemens Vision 1.5 Tesla machine, with VB 33 software. Nephrologists ordered the majority of scans (64.5%). The most common indications were hypertension (91.3%), abnormal renal function (78.3%), other imaging (ultrasound or nuclear scans) suggesting renovascular disease (64.3%), and renal impairment with ACE inhibition (18.3%). 87 scans (71.2%) revealed renovascular abnormalities ('positive'). 73% of scans ordered by nephrologists were positive, compared to 69.7% of scans ordered by other clinicians. 54.5% of patients had three or more risk factors associated with vascular disease, with positive scans in 76% of this group. Localised renal artery stenosis was identified in 65 scans (53.7%). In 40 of these CA and further intervention was not undertaken, mainly due to presence of features of irreversible renal damage, low-grade stenosis or stable clinical parameters. CA was performed in 25 patients, all of whom had moderate-high grade (> 60%) stenosis on MRA. Revascularisation was attempted in 21 of the 25, with technical success in 17. In 22 patients (18.2%) MRA identified non-localised abnormalities, most commonly diffuse arterial disease. None went on to angiography. Summary: Selective use of MRA in "high risk" patients, identified by vascular risk factors or with standard renal imaging, can assist in avoidance of invasive, potentially nephrotoxic CA in up to 80% of cases. Those with treatable lesions can be selected for further intervention.

P109

DELAYED REFERRAL WORSENS LONG-TERM SURVIVAL ON DIALYSIS

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Late referral (LR) is associated with increased morbidity and mortality in the year after starting renal replacement therapy (RRT). We excluded the effect of the first year on RRT (Y1), and examined the effect of LR on the likelihood of transplantation and mortality in subsequent years. We conducted a retrospective analysis using the Australia and New Zealand Dialysis and Transplant Registry for patients who started treatment for ESRD in Australia between 01 April 1995 and 31 Dec 1998. We analysed the likelihood of receiving a transplant, and survival on treatment, for late referred and not late referred groups. Late referral was defined as referral to a nephrologist < 3 months before first treatment. Analysis was adjusted for age, sex, cause of renal disease, number of comorbidities at entry to the program and Aboriginality. We calculated a hazard ratio for death using the Cox's proportional hazards model. Survival time was calculated from the start of the second year on dialysis and patients were censored at transplant, loss to follow-up or death. We excluded 1117 patients who were censored in Y1, leaving 4243 patients available for analysis. The maximum period of follow-up was 4 years (after Y1). 1141 (26.9%) patients were referred late. The patients referred late had a significantly lower likelihood of receiving a transplant (adjusted rate ratio 0.78, 95% CI 0.63–0.95). The post Y1 mortality rate (95% CI) in patients who were referred late was 20 (18–22) per 100 patient years. The mortality rate in patients who were not referred late was 15 (14–16) per 100 patient years. The patients referred late were at significantly increased risk of death in the post Y1 period (adjusted hazard ratio 1.19, 95% CI 1.04–1.35). Including a variable describing treatment modality in the first year on RRT made no significant difference to the analysis. Late referral for ESRD treatment is associated with a lower chance of transplantation and increased mortality even when known early adverse outcomes are excluded. We postulate that LR may be an indicator of sub-optimal pre-ESRD care.

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ACUTE REJECTION IS MORE COMMON AND SEVERE IN LIVE DONOR THAN CADAVERIC DONOR KIDNEY TRANSPLANTS

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Live donors are an increasingly important source of kidneys for transplantation in Australia. The aim of this study was to compare the rate and severity of rejection between patients receiving kidney transplants from live versus cadaveric donors. A retrospective analysis was undertaken of all patients receiving live donor (n = 109) and cadaveric (n = 389) renal transplants at our institution between 1 April 1994 and 31 March 2000. Follow-up was completed on all patients until graft loss, death or 31 May 2001. The baseline characteristics of the live donor and cadaveric groups were similar, except for the proportions of patients receiving first allografts (95% vs 88%, respectively, $p < 0.05$), antibody induction (8% vs 20%, $p < 0.01$) and mycophenolate mofetil (MMF; 60% vs 37%, $p < 0.001$). Acute rejection was observed in 48 (44%) live donor and 108 (28%) cadaveric transplants ($p = 0.001$). Donor type was independently predictive of acute rejection both on logistic regression ($p < 0.001$) and multivariate Cox proportional hazards model analysis (hazard ratio 2.02, 95% confidence interval 1.39–2.92, $p < 0.0001$). Patients receiving live donor transplants were also significantly more likely to receive antibody therapy for rejection (18% vs 9%, $p = 0.006$), independent of age, gender, transplant number, HLA mismatch, sensitisation, induction therapy, delayed graft function or MMF use. However, donor type did not independently influence graft survival, immunologic graft survival or patient survival. In conclusion, live donor kidney transplant recipients had a higher rate and severity of rejection than cadaveric renal transplant recipients. Further consideration of the reasons for this difference and the use of alternative immunosuppressive strategies for live donor transplants are recommended.

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THERAPY WITH mAb TO CD25 BLOCKS FUNCTION OF $CD4^+$, $CD25^+$ T REGULATORY CELLS WHICH MAINTAIN TRANSPLANTATION TOLERANCE

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The regulatory cells that maintain tolerance to transplanted tissue and autoantigens have been identified as a subset of $CD4^+$ T cells that express the IL-2 receptor alpha chain (CD25) (Hall *et al.* J. Exp. Med 1990, 171, 142). These cells are short lived and dependent upon IL-2 for their survival (Pearce et al. Transplantation, 1993, 55, 380). This study examined the role of $CD4^+$, $CD25^+$ T in the maintenance of tolerance induced to PVG cardiac allografts that was induced in DA rats with a 10 day course of anti-CD3 mAb therapy. These rats never reject their graft and after 100 days post transplant accept PVG skin grafts but reject third party grafts. Unfractionated spleen or lymph node cells from these rats with tolerance to the graft transfer tolerance to irradiated host grafted with PVG heart grafts but reject third party grafts. Examination of the sub-populations required to transfer tolerance identified that $CD4^+$ T cell, not $CD8^+$ T cells or B cells transfer tolerance. Separation of $CD4^+$ cells into $CD25^+$ and $CD25^-$ revealed that the $CD25^-$ population restored rejection of PVG grafts and the $CD25^+$ population had no capacity to restore rejection. Thus depletion of the $CD4^+$, $CD25^+$ T cells removed the regulator population revealing $CD4^+$, $CD25^-$ T cells that could restore rejection like naïve cells. Mixing both separated sub-populations did not restore tolerance transfer, however. As we had used MRC Ox39, an anti-CD25 mAb, to separate the cells we examined whether this mAb affected the ability of unfractionated cells from tolerant animals to transfer tolerance. This treatment blocked tolerance transfer. This data is consistent with our *in vitro* data, which showed anti-CD25 mAb blocked survival of regulatory cells. It was concluded transplant tolerance in this model is maintained by a $CD4^+$, $CD25^+$ T regulatory cell whose function is blocked by mAb to CD25. This study raises the question as to the appropriateness of anti-CD25 mAb in the clinic, if transplantation tolerance not requiring long term immunosuppression is to be achieved.

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INCREASED NUMBERS OF Y8 T CELLS IN HEYMANN NEPHRITIS EXPRESSING Vγ6/V81 AND SHOWING A CANONICAL RESTRICTION OF THE CDR3 REGION

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We have reported that the presence of gamma/delta T cells is associated with kidney damage in human IgA nephropathy and in a rat model of adriamycin (ADR) induced nephropathy. We have also shown that these T cells use a restricted set of gamma/delta T cell receptor (TCR) genes. This study examines whether T cells infiltrating the kidney also use a restricted set of gamma/delta T cell receptor genes in Heymann nephritis (HN), a rat model of autoimmune mediated glomerulonephritis.

HN was induced in Lewis rats by immunisation with renal tubular antigen (Fx1A) in CFA. Kidneys, spleen and lymph nodes were collected 8 and 12 weeks after immunisation. T cells were isolated from the kidney using a gradient separation method. Flow cytometry analysis (FACS) was used to determine the percentage of gamma/delta T cells as a proportion of the total number of CD3 + T cells. TCR Vgd repertoire was measured by RT-PCR and sequencing was used to characterise the diversity of the CDR3 region of these receptors. Cytokine gene expression was measured by semiquantitative RT-PCR.

FACS of lymphocyte subpopulations using anti-CD3 and anti-gdTCR antibodies showed that gamma/delta T cells as a proportion of CD3 + cells were significantly increased in HN kidneys (6.9 +/– 2.9%) but not in lymph nodes (1.6 +/– 0.35%), ($n = 8$, $p < 0.01$). Analysis of the kidney TCR Vgd repertoire showed that these cells predominantly expressed Vg6/Vd1 genes. Sequencing analysis of the Vg6/Vd1 junctional region showed that they used canonical sequences identical to those expressed on fetal thymocytes. RT-PCR for cytokine gene expression showed that gamma/delta T cells from the kidneys expressed significantly higher levels of IL-4 ($p < 0.05$) and IL-5 ($p < 0.05$) and significantly lower levels of IL-2 ($p < 0.05$) compared to gamma/delta T cells from the lymph nodes.

These results demonstrate that the majority of gamma/delta T cells in the HN kidney use an invariant, canonical Vg6/Vd1 TCR—exactly the same gamma/delta TCR we have previously described in the rat ADR kidney. These canonical Vg6/Vd1 T cells may recognise an unknown common ligand expressed in the kidney in response to a wide variety of causes of chronic inflammation.

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IL-4 AND IL-5 THERAPY INHIBITS HEYMANN NEPHRITIS (HN)

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Active HN induced by immunization of Lewis rats with renal tubular antigen (RTA) in complete Freund's adjuvant (CFA), is a model of human membranous nephritis. IgG and complement (C) deposits in glomeruli and the onset of proteinuria is preceded by cytotoxic $CD8^+$ T cell and macrophage infiltrate in the glomeruli. The dominant T cell response is Th1 (mainly IL-2, IFN-γ TNF-β) and the complement fixing isotypes (IgG2a and IgG2b) being induced. IL-4 and IL-5 cytokine of Th2 cells, one of the T cell subset that modulate Th1 responses was examined in this study of HN. Rat rIL-4 and rIL-5 prepared from transfected CHO-K1 cells were administered for 10 days (30 000 unit/day, i.p) at the time or 4 weeks post-immunization, when the $CD8^+$ T cell response develops immediately prior to the onset of proteinuria. Early therapy with rIL-4 or rIL-5 did not prevent proteinuria. rIL-4 but not rIL-5 therapy induced high titers of Th2 dependent IgG1 and cytokine mRNA for IL-4 and IL-5. Late treatment with either rIL-4 or rIL-5 delayed onset of proteinuria, with levels at 8 and 12 weeks not different to controls immunized with CFA negative controls, but significantly different to HN positive disease controls. Urine protein at 12 weeks was 51+8 mg/day in HN, 16±13 in IL-4 treated, 14+2 in rIL-5 treated and 2±1 in CFA controls (n=5 per group). This experiment was repeated twice with similar results. There was no difference in serum anti-RTA Ab (IgG or isotypes) at 6, 8 or 12 weeks. Glomerular IgG and C3 deposition was similar in all HN groups, but the $CD8^+$ T cell and macrophage infiltrate was reduced in rIL-4 and rIL-5 treated groups compared to HN. This was accompanied by a lower level of mRNA for Th1 cytokines (IFNγ and TNF-β) glomeruli isolated from both rIL-4 and rIL-5 treated rats. This study shows IL-4 and IL-5 can inhibit Th1 responses that mediate glomerular injury in HN.

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GLOMERULAR T CELL V BETA REPERTOIRE IN EXPERIMENTAL AUTOIMMUNE GLOMERULONEPHRITIS

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Experimental Autoimmune Glomerulonephritis (EAG) is a model of human Goodpasture's disease induced in susceptible strains of rats by a single intramuscular injection of collagenase-solubilised rat glomerular basement membrane in adjuvant. This induces severe proteinuria and crescentic nephritis at 4 weeks. Previous work has shown this to be a T cell dependent disease (1). The aim of this work was to investigate the T cells infiltrating the glomeruli in this disease model using reverse transcription PCR, Complementarity Determining Region 3 (CDR3) spectratyping and sequencing. EAG was induced in Wistar Kyoto rats by standard methods. The kidneys were perfused with saline and the glomeruli separated by a sieving method. RNA was extracted and reverse transcribed to cDNA. Polymerase chain reaction using primers for 20 T cell receptor V beta genes was used to further examine the cDNA. This showed the overexpression of an average of two V beta families in each kidney analysed. Overexpressed families varied from animal to animal. CDR3 spectratyping of Fam-labelled PCR product showed widespread restriction of multiple V beta families compared to matching splenic samples. Two V beta families (BV16 and BV10) showed a common spectratype band across six of seven animals. Three families showed a further common band across five of seven animals. Sequencing is being performed to further investigate these spectratypes. Restricted spectratypes shared between animals suggest that oligoclonal T cells are infiltrating the glomeruli and that similar clones may be present in different animals. These results suggest that T cells recognising specific antigens infiltrate the glomerulus in the course of EAG.

1. Reynolds J, Tam FWK, Chandraker A, Smith J, Karkar AM, Cross J, Peach R, Sayegh MH, Pusey CD. CD28-B7 blockade prevents the development of experimental autoimmune glomerulonephritis. J. Clin. Invest. 105:643–651, 2000.

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CHEMOKINES ARE IMPORTANT INITIATORS OF CELLULAR XENOGRAFT REJECTION

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Objective: Models of the *cellular* phase of xenograft rejection based on non-vascularized tissue grafts such as fetal pig pancreas (FPP), are CD4+T cell and macrophage dependent. We investigated how these effector cells may be recruited by performing time course studies of intragraft chemokine and chemokine receptor gene expression during rejection and after short-term costimulation blockade.
Methods and Materials: FPP fragments were transplanted under the renal capsule of C57Bl/6 mice. Islet isografts were controls for the surgical procedure. Treatment animals received CTLA-Fc (500 ug day 2) and anti-CD40L (500 ug days 0,2,4&6). Gene expression of 9 chemokines and 6 chemokine receptors were examined by multi-probe ribonuclease protection assays (RPA) and cellular infiltration was examined by confocal microscopy.
Results: Rejecting xenografts showed enhanced expression of MIP-1B on day 2 corresponding with initial macrophage appearance, and MIP-1B, MCP-1, RANTES, IP-10, and lymphotactin on day 4 corresponding with initial CD4+ T cell appearance and further macrophage accumulation. Chemokine receptor expression was consistent with the ligand profile with CCR1, CCR2, and CCR5 upregulated on days 2&4. Eotaxin, MIP-1A, MIP-2, TCA-3, and CCR1b, CCR3 and CCR4 expression did not differ from isograft controls. This chemokine and chemokine receptor data was consistent with a Th 1 type response. Costimulation blockade resulted in 100% graft survival at 100 days and was associated with suppression of the early chemokine gene expression seen in rejecting xenografts and a reduction in early inflammatory cell infiltrate.
Conclusions: This selective chemokine/receptor profile was consistent with the temporal appearance of macrophages and CD4+T cells. Short-term costimulation blockade resulted in indefinite FPP survival and was associated with abrogation of the early chemokine gene response and cellular infiltrate suggesting these molecules are important early mediators of the leukocyte infiltrate in FPP xenograft rejection.

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IS SIZE AT BIRTH INVERSLEY RELATED TO RISK OF HYPERTENSION IN LATER LIFE?

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Objective: To study the effect of size at birth on blood pressure (BP) in later life.

Bakcground: The fetail origins hypothesis suggests that fetal under-nutrition during sensitive periods of rapid growth induce permanent changes in the structure and function of fetal organs, such as the kidneys. It has been shown that low birth weight is associated with high BP, both in children and adults. Explanations for this association include a direct effect of adverse intra-uterine environment on the cardiovascular system or as a sequelae of excess postnatal "catch-up" growth. More recently it has been proposed that there is a direct effect on the number of nephrons formed in utero in response to a inadequate intra-uterine environment. There is evidence for the third explanation from animal experiments.

Methods: A cross sectional community based study was carried out in a remote coastal Aboriginal community. All participants had height, weight and blood pressure measurements. Birth parameters were obtained from clinic and hospital records.

Results: There were 409 participants (215M; 194F) with a mean age 20.8 years (range 4.4–42.4 years). There wer 226 adults and 183 children. BP measurements were higher in men, and in those with higher BM1 Systolic and diastolic BP showed significant inverse correclation with birth weight and birth length. When corrected for BM1, age and sex, the correlation between diastolic BP and birth weight ($p=0.038$) and birth length persisted ($p=0.003$) but was no longer significant for systolic BP. There is an increase of 1.8 mmHg of diastolic BP of every kg decrease in birth weight and of 0.5 mmHg for every cm decrease in birth length (adjusted for age, sex bmi).

Conclusion: We have previously shown a relationship between kidney volume and size at birth, so we propose that the inverse relationship of birth weight and length with BP is probably due the effect of an adverse intra-uterine environment causing a lower of nephrons being formed which are more susceptible to insults in later life and thus result in the development of hypertension and renal failure.

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AMBULATORY BLOOD PRESSURE MONITORING IN CHRONIC RENAL FAILURE PATIENTS RECEIVING REGULAR HAEMODIALYSIS

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To examine the cardio-vascular burden of our hemodialysis (HD) patients, we performed ambulatory blood pressure monitoring (ABPM) in 16 hypertensive (HT) and 14 normotensive (NT) HD patients, during dialysis and the subsequent 24 hour. Echocardiography (ECHO) was performed on a non-dialysis day. Conventional blood pressure (BP) was measured sitting on arrival at the HD unit and 10 minutes thereafter, with sphygmomanometer (SPM) and dynamap (DNM). Correlations between BPs and cardiac parameters shown by ECHO were compared in HT and NT patients. Male/female ratio, body mass index (BMI), duration of HD, Hematocrit (Hct), interdialytic weight gain (IDWG) between HT and NT were statistically. not different. Mean BP during HD were not statistically different from mean BP during ABPM, both total and daytime BP, in HT as well as NT patients. Likewise BP taken by SPM, DNM and ABPM were also not statistically different. In total only 5 patients were classifiable as dippers, 3/16 in HT and 2/14 NT. LVMI in HT was higher than NT, although did not reach statistical significance. Only one out of 14 HT and 4 out of 14 NT patients were classifiable as not having left ventricular hypertrophy (LVH). Ejection fraction in HT was lower than in HT ($p < 0.01$). LVMI correlated positively and significantly with all ABPM readings, except with nocturnal systolic BP, and negatively with Hct in HT. Ejection Fraction (EF) correlated negatively and significantly with diastolic BP, total, daytime and night time, and positively with Hct in HT but no such significance were found in NT. Our findings revealed the heavy cardio-vascular burden of our HD population, even in NT, most likely due in part to the loss of dipping property.

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RAPID TREATMENT OF SEVERE HYPERTENSION IN PREGNANCY

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Background—The withdrawal of Nifedipine capsules, proven to be effective in the management of severe hypertension in pregnancy, left only intravenous hydralazine for treatment of acute severe hypertension in pregnancy.
Aim—To determine whether Nifedipine tablets lower blood pressure as safely and effectively as Nifedipine capsules in severely hypertensive pregnant women.
Methods—Pregnant women in the second half of their pregnancy with severe hypertension (systolic BP ≥ 170 mmHg and/or diastolic BP ≥ 110 mgHg) were enrolled in a prospective randomised trial (PROBE design) to receive either Nifedipine 10 mg capsules ($n = 31$) or (slower onset) 10 mg tablets ($n = 33$). Blood pressure, pulse rate and CTG monitoring were undertaken throughout the subsequent 90 minutes and a second dose of the same treatment was administered after 45 minutes if severe hypertension persisted. "Successful" treatment was a subsequent blood pressure 110–169/80–109 mmHg and "unsuccessful" treatment included relative hypotension, persistent severe hypertension or fetal distress.
Results—Both groups were of similar age, gestation and parity and lasted a mean of 4 days from study until delivery. A similar proportion achieved 'successful' outcomes (77% capsules vs 81% tablets, $p = 0.56$). Twice as many women receiving tablets (28%) required a second dose after 45 minutes, $p = 0.05$, though fewer had hypotensive episodes (9% tablets vs 37% capsules, $p = 0.03$). After 90 minutes BP had fallen significantly more in those receiving capsules (28/19 mmHg) than those receiving tablets (21/13 mmHg), $p = 0.03$. The groups were similar in the proportion delivered for uncontrolled blood pressure or other maternal complications, and had babies of similar birth weight.
Conclusions—Nifedipine tablets lower blood pressure to an acceptable range within 90 minutes in 80% of women. Although one in four women needed a second tablet to achieve this result, far fewer had episodes of relative hypotension. Nifedipine tablets are a safe and effective alternative to Nifedipine capsules for the acute treatment of severe hypertension in pregnancy.

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INTERLEUKIN-10 GENE PROMOTOR REGION ABNORMALITIES IN PREECLAMPSIA

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A lack of placental interleukin-10 has been identified in patients with preeclampsia. This confers a greater inflammatory response in thes placentas compared with normal. In order to assess the alteration in material circulating cytokines and interleukin-10 promoter region was examined in placental tissue and maternal blood DNA. Methods: Mutagenically-separated PCR was used to identify the single nucleotide polymorphism A : G at 5′ upstream position—1082 of the IL-10 gene promoter region. This system relies on the differential amplification of a segment arising from the nucleotide of interest with primers designed to bind to each nucleotide (A or G). Presence of the *A allele is associated with a decrease in interleukin-10 production. The frequency of the two alleles will be determine din maternal blood, and in placental tissue at delivery. The AB1310 capillary electrophoresis was used to detect fluorescence of the PCR product. Women with preeclampsia ($n = 25$), were matched for age and parity with normal pregnant women ($n = 14$) frequency between groups was by Chi sqared test. Results: There was no significant difference in gene frequency between the patients with preeclampsia and gestational hypertension or normal pregnancy.

Genetype	GG	AA	AG	P
Normal Pregnancy	21%	42%	37%	NS
Gestational hypertension	17%	50%	33%	NS
Preeclampsia	17%	33%	50%	NS
Normal Population femailes (18–35 years)	17%	33%	50%	

Conclusion. In a small sample of preeclamptic patients, a deficiency of interleukin-10 is not explained by the G single nucleotide polymorphism at position—1082. Identification of other promoter region insertions may be necessary to identify an inherited component to maternal preeclampsia.

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NEPHRON NUMBER, RENAL FILTRATION SURFACE AREA AND BLOOD PRESSURE IN F_2 HYBRIDS OF A SPONTANEOUSLY HYPERTENSIVE RAT/WISTAR-KYOTO RAT CROSS

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The objectives of this study were to determine whether there are direct relationships between: (1) glomerular number and/or size and the level of blood pressure in the adult rat; and (2) total renal filtration surface area and the level of adult blood pressure. F_2 offspring from a spontaneously hypertensive rat/Wistar-Kyoto rat cross, which develop differing blood pressures later in life due to random genetic mixing, were used. Systolic blood pressure was measured by tail-cuff sphygmometry from 5 to 15 weeks of age. Using unbiased stereological techniques total glomerular number, glomerular size, total length and surface area of glomerular capillaries and total renal filtration surface area were determined at 15 weeks of age. As well as looking for correlations using the entire population, rats were also genotyped into 3 groups based on their SHR renin allele inheritance. At 15 weeks of age a range of mean arterial pressures (124 mmHg to 160 mmHg) and nephron numbers (21 574 to 37 501 glomeruli/kidney) was observed in the F_2 generation. Despite these ranges, no correlation between blood pressure in the adult rat and nephron number and/or total renal filtration surface area was found. Although higher blood pressures were observed for rats inheriting the SHR renin allele, no relationships were found between nephron number and/or total renal filtration surface area and genotype in the three genotypic groups. Therefore, the development of hypertension in these F_2 rats relies on mechanisms other than the reduction in nephron number and/or a reduction in total renal filtration surface area.

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HYPERTENSION AS A DETERMINANT OF SURVIVAL FOR DIALYSIS PATIENTS

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Previous studies of the risks of hypertension for dialysis patients have yielded conflicting results. The aim of this study was to investigate, in a home dialysis population with low rates of diabetes and antihypertensive drug use, whether blood pressure (BP) was an independent risk factor for survival. We retrospectively analysed the outcome of 168 consecutive patients (94 male, 88% Caucasian), aged 48 years (sd 16), who began home HD (n=124) or home CAPD (n=44) between 1st January 1985 and 31 December 1994. Only 4.7% of patients took antihypertensive drugs while on dialysis. The patients were followed to 31 December 1998, with the primary outcome being all cause mortality. Censoring events were transplantation, transfer to another centre and treatment modality change. The Cox proportional hazard model was used with baseline predictors. Seventy-one patients died and the median survival (both modalities) was 4.2 yr. Mean BP at start of dialysis predicted survival on its own (p=0.0009) and in the joint Cox model (p=0.047). Other significant predictors in the joint model were age (10 year increase, relative hazard (RH)=1.55, p=0.0008), albumin (10 g/l decrease, RH=2.05, p=0.007), diabetes (RH=3.42, p=0.015) and peripheral vascular disease (RH=2.19, p=0.02) but not dialysis modality (RH=1.63, p=0.13). High and low mean BP at the start of dialysis were associated with the highest mortality. Amongst home dialysis patients, most of whom did not require antihypertensive drugs, hypertension was a risk factor for survival and patients with mid-range BP survived longest.

P122

OSTEOPONTIN-INDUCED MACROPHAGE RECRUITMENT IN ADVANCED EXPERIMENTAL DIABETIC NEPHROPATHY

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Macrophages have been implicated in the pathogenesis of progressive kidney disease, although their role in diabetic nephropathy (DN) is unclear. In the present study macrophage infiltration and the expression of the macrophage chemotactic protein, osteopontin (OP) in experimental DN was examined. The effects of blockade of the RAS was also examined. Diabetes was induced with streptozotocin (STZ) in the transgenic Ren-2 rat, a diabetic rodent model which develops features similar to human DN. Rats were randomized to control (C), diabetic with or without the ACE inhibitor, perindopril (D, DP) and sacrificed at 12 weeks. OP mRNA and protein were localized by in situ hybridization and immunohistochemistry and quantitated using image analysis. Macrophages were detected by rat ED-1 positive immunostaining.

	OP mRNA (ROD)	TID (PA)	MAC (cells/mm2)
C	1.7±0.8	8.1±1.1	1±1
D	20.5±7.0*	18.1±3.5*	50±15*
DP	1.1±0.1†	3.8±0.70†	11±5†

*p<0.01 v C, †p<0.01 v D. OP, osteopontin; MAC, macrophages; TID, tubuolointerstitial disease; ROD, relative optical density; PA, proportional area with tubuolointerstitial changes.

OP expression and associated macrophage infiltration is increased in diabetes and decreased with ACE inhibition. These findings suggest that OP expression and macrophage accumulation may play a role in the tubulointerstitial injury in diabetic nephropathy and that inhibition of OP expression may underlie the renoprotective effects of RAS blockade.

P123

VEGF GENE EXPRESSION IN PROTEINURIC RATS

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Vascular endothelial growth factor (VEGF) is a permeability-enhancing, endothelial cell mitogen, implicated in microvascular exudation. Its expression in vascular smooth muscle cells is increased by angiotensin II (AngII). However, the role of VEGF in glomerular proteinuria and its AngII-responsiveness in the kidney are uncertain. 24 rats were randomized to three groups (n=8/group): control, subtotal nephrectomy (STNx) and STNx+ACEi (perindopril). Rats were sacrificed at 12 weeks post surgery. Expression of VEGF mRNA was assessed by quantitative in situ hybridization and glomerulosclerosis (GS) was assessed by scoring PAS stained sections. STNx rats developed hypertension, proteinuria and declining GFR in association with reduced VEGF expression.

	VEGF (WKROD)	VEGF (% glom area)	Albuminuria (mg/24 hs)
Control	35.5±3.5	0.68±0.2	21×/÷1.1
STNx	23.2±1.4*	0.13±0.04*	328.4×/÷1.2*
STNx+ACEi	43.6±3.1#	0.5±0.1#	171.5×/÷0.90#

VEGF mRNA expressed as relative optical density in whole kidney (WKROD) and % glomerular area occupied by autoradiographic grains. *p<0.01 v control, #p<0.01 v STNx. Glomerular VEGF expression and GS score in STNx rats was closely correlated (R=0.64, p=0.001).

These findings suggest that despite its permeablility-enhancing properties and stimulation by AngII at other sites, VEGF is unlikely to mediate proteinuria in STNx. Furthermore, VEGF mRNA was increased by ACEi. VEGF may be important for the maintenance of glomerular capillary integrity and that reduction in expression, possibly as a consequence of podocyte loss is associated with GS. ACEi may be renoprotective by preserving podocyte VEGF expression.

P124

GLUCOSE AND ALBUMIN INDUCED ENHANCEMENT OF NF-kB-SPECIFIC PROTEIN EXPRESSION IN HUMAN RENAL PROXIMAL TUBULAR CELLS

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It has been well established that the proximal tubule is exposed to an excessive load of filtered glucose and albumin in diabetic nephropathies. Several studies support a role of glucose and albumin in the pathogenesis of renal interstitial inflammation and fibrosis. The transcription of numerous inflammatory or fibrotic mediators such as IL-1b or TNF-a is mediated by the nuclear factor [kappa]B (NF-kB). Thus we tested the hypothesis that NF-kB could be involved in glucose- or albumin-induced renal interstitial inflammation and fibrosis in human cells. Confluent monolayers of human primary proximal tubular cells (PTC) and the human proximal tubular cell line IHKE-1 were exposed to bovine serum albumin (BSA) at 100 and 1000 mg/l (physiological and pathophysiological concentrations that are found in tubular fluid) in media containing 5 mM or 25 mM glucose (physiological or pathophysiological glucose concentration) for 12, 24, and 48 hrs. Control exposures were done in albumin free media (containing 5 mM glucose). The NF-kB-specific protein expression (p50 and p65 subunit) was analysed by Western blot. 25 mM glucose exposure or albumin exposure both induced a significant increase in NF-kB-specific p50 and p65 expression in both, PTC and IHKE-1 cells as compared to control after 12, 24, and 48 h exposure (PTC: n=6, p<0.05; IHKE-1: n=5, p<0.05). Simultaneous 25 mM glucose and albumin (1000 mg/l) exposure had no additive increasing effect on NF-kB-specific protein expression. These data shown, that glucose or albumin exposure induces an increase in NF-kB-specific protein expression in human renal proximal tubular cells. Thus glucose- or albumin-induced NF-kB activation may be involved in glucose- or albumin-associated inflammatory or fibrotic renal pathomechanisms.

P125

OXIDATIVE STRESS AND HDL FUNCTION IN END STAGE RENAL DISEASE

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Background: Increased oxidative stress and reduced HDL levels or function may contribute to excess atherosclerosis in End Stage Renal Disease (ESRD).
Study objectives: To determine if in patients with ESRD vs healthy controls (1) plasma protein carbonyls (a measure of oxidative stress) are increased and (2) if HDL quantity and quality is reduced.
Methods: Fasting blood was drawn from 20 subjects per group. Protein carbonyls were measured by ELISA, the lipoprotein profile by enzymatic assays and the Friedewald equation, activity of paraoxonase (PON) a HDL associated antioxidant enzyme measured by spectroscopic assays of hydrolysis of paraoxon and phenylacetate. HDL efficacy in removing lipid peroxides was assessed by measuring lipid peroxides in an oxidized cell membrane HDL admixture. $\alpha < 0.05$ was taken as significant.
Results: Protein carbonyls were increased in ESRD (0.13 (SD 0.057)) vs controls (0.046 (SD 0.015). PON activity did not differ between groups (ESRD 13.3 vs control 15.2; p = 0.5). HDL levels tended to be lower in ESRD (1.22 (SD 0.44) vs 1.505 (SD 0.4)) but ESRD HDL was more effective in lowering lipid peroxides 70.92% vs 42.44%. Efficacy of lipid peroxide removal correlated inversely with serum HDL levels, but not with PON activity.
Conclusions: Oxidative stress is increased in ESRD. While HDL levels tend to be lower in ESRD its efficacy in removing lipid peroxides is greater that that from healthy subjects. This may be a compensatory effect and cannot be attributed to increased PON activity. The effects of reduced oxidative stress and increased HDL levels in ESRD and vascular disease warrants investigation.

P126

TRANSLOCATION OF CAVEOLIN-1 IN RENAL ISCHAEMIA-REPERFUSION INJURY

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Caveolae and their proteins, the caveolins, transport macromolecules and compartmentalise signaling molecules, actions that are needed in repair after injury. The caveolins have been localised in renal tissue, but their role in the pathogenesis of renal disease is not known. In this study, spatiotemporal links between caveolins 1 and 2 and tubular epithelial and vascular pathologies of ischemic acute renal failure were investigated using an *in vivo* rat model (30 minute bilateral ischemia, reperfusion from 4 hr to 1 week), and cell lines (renal tubular epithelial cells or arterial endothelial cells subjected to injury modeling for renal ischemia-reperfusion). Immuno-blots (whole cell or membrane and cytosol fractions) and immuno-localisation of caveolin proteins were analysed. Caveolin-1 was expressed in vascular endothelial cells, glomerular capillary network and in apical and basolateral membranes of distal tubular epithelium in healthy kidneys. Caveolin-2 had similar, but weaker, vascular localisation. In ischaemia-reperfused kidneys, additional punctate cytoplasmic localisation of caveolin-1 was seen in proximal tubules that were losing basement membrane adhesion or were apoptotic. *In vitro*, only caveolin-1 was found in both cell types, before and after treatment. Treatments induced translocation of caveolin-1 from its major site (plasma membrane) into cytoplasmic sites. Whole cell expression levels did not alter. The protein remained membrane-bound within the cytoplasm. It localised intensely around bodies of neutral lipid and also within apoptotic cells. The results are suggestive of a means of transport of molecules that may either act for repair of ischaemia-reperfusion damaged cells or may be toxic to the cells.

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P127

APOPTOSIS AND MAPK SIGNALLING IN RENAL EPITHELIAL, FIBROBLAST AND VASCULAR ENDOTHELIAL CELLS AFTER FIBROGENIC STIMULI

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Variation in the microenvironment of the kidney during progressive healing and fibrosis after injury affects cellular survival and involves various intracellular signal transduction pathways. In this study, differences in survival of renal epithelial (NRK52E), fibroblast (NRK49F) cells and vascular endothelial cells (BAEC) were analysed after challenge with oxidative stress or the fibrogenic growth factors transforming growth factor-β1 (TGF-β1, 10 ng/ml) and tumour necrosis factor-α (TNF-α, 50 ng/ml) over 18–20 hrs. Specific members of the mitogen activated protein kinase (MAPK) family were studied. Apoptosis increased in NRK49F cells exposed to 1.0 mM H_2O_2 (38.2 ± 8.5% v controls at 6.2 ± 0.9%, $P < 0.05$). Similar results were observed in NRK52Es and BAECs, where % apoptosis rose from 2.5 ± 0.5 (control) to 10.4 ± 1.8 ($P < 0.05$) and from 6.2 ± 1.2 (control) to 13.5 ± 3.5 ($P < 0.05$), respectively. Treatment with TGFβ1 or TNFα did not significantly alter apoptosis in NRK49Fs and NRK52Es. In BAECs, TNFα increased apoptosis (6.2 ± 1.2 versus 16.2 ± 5.9, $P < 0.05$). H_2O_2-induced apoptosis in NRK49Fs was associated with activation of extracellular-related protein kinase 1/2 (ERK1/2) and was inhibited by the MAPK kinase (MEK) inhibitor, PD98059 (20 μM) (38.2 ± 8.5 reduced to 13.5 ± 4.6, $P < 0.05$). However, MEK inhibition did not alter H_2O_2-induced apoptosis levels in NRK52E and BAEC cells. TNFα-induced apoptosis of BAECs was associated with activation of stress activated protein kinase (SAPK). Thus, the susceptibility of cells to different stresses and the signalling pathways induced during healing and fibrosis after injury are diverse, complex and dependent on the cell type in the heterogeneous population of the kidney.

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P128

DIABETES ACCELERATES RENAL INJURY IN THE APO E DEFICIENT MOUSE MODEL OF ATHEROSCLEROSIS

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Atherosclerosis and diabetes are each important causes of morbidity and mortality in the developed world. We have investigated the interaction between these conditions by analysing kidney morphology in mice that are atherosclerotic due to lack of apolipoprotein e (apo) and apo E knockout mice made diabetic. Diabetic Apo e-/e- mice display hypercholesteremia with high LDL-cholesterol and low plasma triglycerides. Previous studies have shown that double deficient mice for Apo E and the endothelial nitric oxide synthase (eNOS) develop accelerated kidney damage, glomerular lipid deposition, and calcification.

12 apo e-/e- mice were made diabetic at 6 weeks of age with streptozotocin injections intraperitoneally (50 mg/kg body weight, daily for 6 days). 6 apo e-/e- mice served as controls. All streptozotocin injected animals were confirmed to be diabetic (glucose > 15 mmol/l). The mice were then randomised to receive the ACE inhibitor perindopril in the drinking water or placebo for 20 weeks. At 20 weeks of age atherosclerosis is well established. Baseline parameters of all mice at 20 weeks are summarized Table 1:

	apo e-/e-	diabetic apo e-/e-	diabetic apo e-/e- + Perindopril
Body weight(g)	32	22*	23
Glucose (mmol/L)	10.4	33.1*	29.9
GS-index median (range)	2.4 (2–3)	3.4 (3–4)≠	3 (2–4)

*$p < 0.01$ versus apo e-/e-: ≠$p = 0.03$ versus apo e-/e-.

Glomerulosclerosis index (GS) was assessedin HE stained sections. The diabetic Apo e-/e- mice showed significantly more glomerulosclerosis and tubular dilatation than the non-diabetic apo e-/e- mice which was also accompanied by inflammatory cellular infiltration. Treatment with the ACE inhibitor perindopril ameliorated tubular dilatation and to a lesser degree glomerular injury in the diabetic apo e-/e- mice.

Diabetes in the apo e-/e- mouse is associated with accelerated renal injury and these changes are ameliorated by ACE inhibitor treatment. Further studies are in progress to investigate the mechanisms involved with renal structural injury in this model of atherosclerosis and diabetes.

P129

THE ROLE OF HB-EGF IN EXPERIMENTAL ISCHAEMIC ACUTE RENAL FAILURE

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In models of acute renal ischaemia, HB-EGF is strongly up-regulated, although its function in this disease is unknown. In this study HB-EGF was examined in a model of ischaemic acute renal failure (iARF). The monoclonal antibody DE10, which blocks the function of HB-EGF, was administered to uni-nephrectomised rats that had undergone 45 minutes of ischaemia in the remaining kidney. Control rats received an isotype-matched antibody. In the control rats (n=4) serum creatinine levels peaked at day 2 and fell to pre-ischaemic levels by day 9. The DE10-treated animals (n=5) showed a similar rise in serum creatinine, however, the values at days 2 and 3 were significantly lower compared with the controls (0.25 vs 0.36 mmol/L at day 2; $p<0.01$ and 0.21 versus 0.31 mmol/L at day 3; $p<0.01$). PAS staining of control and DE10-treated kidneys at day 4 showed less obstruction of tubules with necrotic cells in the DE-10 treated group. These results suggest that increased expression of HB-EGF contributes to tubular obstruction in iARF, probably through its known roles in cell-cell and cell-matrix adhesion and anti-apoptotic functions. In an *in vitro* model of tubular injury, NRK-52 E cells were treated with mercuric chloride and analysed for HB-EGF expression and Annexin V, a marker for early apoptotic cells. Pre apoptotic cells express high levels (50-fold) of HB-EGF compared to untreated cells and the cells are strongly positive for Annexin V. In conclusion, this data shows that the increased expression of HB-EGF in acute renal failure promotes, rather than retards the loss of kidney function. This may be due to the anti-apoptotic function of HB-EGF causing increased blockage of renal tubules by viable, desquamated tubular cells.

P130

INCREASED BIOLOGICALLY ACTIVE TGF-β1 AND OVEREXPRESSION OF PDGF IN RENAL BIOPSIES OF HUMAN DIABETIC NEPHROPATHY

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TGF-β1 has been implicated in the pathogenesis of diabetic glomerulosclerosis and tubulointerstitial fibrosis. However, other prosclerotic growth factors such as PDGF may also contribute to this process. The present study sought to; (1) determine gene expression of PDGF, and (2) assess both gene expression and the biological activity of TGF-β1 in human diabetic nephropathy (DN). Gene expression of PDGF-A, PDGF-B and TGF-β1 were measured in renal biopsy tissue samples from human DN (n=7) and from normal nephrectomy specimens (n=4) using competitive RT-PCR. Immunohistochemistry for PDGF-A, PDGF-B, and TGF-β-inducible gene-H3 (βig-H3), a marker of biologically active TGF-β1, was also performed, and the latter analysed by quantitative image analysis (AIS; Ontario, CA).

	PDGF-A#	PDGF-B#	TGF-β1#	βIG-H3##
DN	7 857	9 325	49 868	10.9 (±1.9)*
	(5 328, 11 584)*	(4 493, 19 353)*	(30 242, 82 207)*	5.0 (±1.4)*
Neph	346	1 046	774	0.06 (±0.02)
	(243, 492)	(771, 1 420)	(498, 1 202)	0.8 (±0.2)

#Copy no./ngm RNA; geom. mean (95th centile CI). ##Percentage area, mean (±sem), glomerulus, tubulo-interstitium. * $p<0.001$, DN v nephrectomy. In addition to a marked increase in mRNA, increased immunostaining for PDGF-A, -B, and βig-H3 was noted in glomeruli and tubulointerstitium of DN, but not in nephrectomy tissue. These findings implicate PDGF in the pathogenesis of diabetic nephropathy and show that TGF-β1 is expressed in its biologically active form.

P131

PHENOTYPIC ALTERATIONS IN TUBULAR EPITHELIUM IN A RAT MODEL OF AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

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The present study assessed weather phenotypic alterations in tubular epithelium develop in a Han:SPRD rat model of human autosomal dominant polycystic kidney disease (ADPKD). By Northern analysis, a-smooth muscle actin (SMA) and vimentin mRNA expression were markedly increased in both male and female homocystic (Cy/Cy) animals at 2 and 3 weeks of age compared to either normal (+/+) or heterozygous (Cy/+) age-matched animals. There were no differences between normal and heterozygous rats at these early time points; however, by 8 and 24 weeks, a-SMA and vimentin mRNA expression were markedly increased in heterozygous versus normal rats. a-SMA has been reported as a marker for myofibroblast transformation. In addition to the detection of a-SMA in the widened interstitial region surrounding the cystic tubules of homozygous and heterozygous rats, there was a pronounced expression of a-SMA protein and mRNA by immunohistochemistry and in situ hybridization, respectively, in the epithelial cells lining the dilated cystic tubules. Immunolabeling for vimentin demonstrated an intense epithelial cell staining pattern in the dilated tubules from homozygous rats. Transforming growth factor (TGF)-b1 may be an inducer of this epithelial-mesencymal transformation process. At 2 and 3 weeks of age in both genders, TGF-b1 mRNA expression was considerably greater in homozygous animals versus either normal or heterozygous rats. At 8 and 24 weeks of age, male heterozygous rats exhibited an up-regulated TGF-b1 mRNA expression versus normal rats. This study provides evidence for a novel process of epithelial cell transformation to a more fibroblast-like phenotype in the kidneys of Han:SPRD rats.

P132

PROTECTION OF BCL-XL TRANSFECTED RENAL EPITHELIAL CELLS DURING OXIDATIVE STRESS

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Ischaemia-reperfusion is a major cause of renal tubular epithelial cell death in acute renal failure. Cells are first deprived of oxygen and then bombarded by free radicals. Our work focuses on the protective role of the Bcl-2 gene family during this oxidative cell stress. Previous studies have indicated that surviving, ischaemia-reperfusion injured, renal tubular epithelial cells are protected by altered expression of anti-apoptotic Bcl-X_L and, principally, by its translocation to the mitochondria. In the present study, Madin Derby canine kidney (MDCK) distal tubular epithelial cells grown on coverslips were transiently transfected with vectors containing green fluorescent protein (GFP), GFP/Bcl-X_L or GFP/Bax. They were then exposed to 5 mM hydrogen peroxide (H_2O_2) for 1 h. Apoptosis was assessed using morphology, verified using *in situ* end labelling and DAPI nuclear staining, and quantified as a percentage of total cells in a ×200 microscope field. H_2O_2-treated cells transfected with GFP/Bcl-X_L showed significantly less apoptotic cells per field (2.4±0.6%) compared to the GFP control (5.4±2.7%, $p<0.01$) and GFP/Bax (4.2±1.5%, $p<0.01$). Cells transfected with GFP/Bcl-X_L were also less prone to loss of adhesion from the coverslip after treatment, with significantly more cells present per field (235.4±41.1) compared to the GFP control (61.8±16.6, $p<0.001$). The results indicate that Bcl-X_L gives cellular protection against death and also stabilises the cells during oxidative stress. The protection may allow them to synthesise regenerative growth factors, further assisting in their survival.

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P133

BCL-2 EXPRESSION IN PROGRESSIVE VERSUS NON-PROGRESSIVE IGA NEPHROPATHY

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The pathological mechanisms that mediate progressive renal injury in some patients with IgA nephropathy (IGAN) and not in others remain poorly experimental proliferative glomerulonephritis as well as contributing to the development of glomerulosclerosis and tubular atrophy. This study examines the interplay between progressive (P) and non-progressive (NP) IGAN and proteins that regulate apoptosis, in particular the anti-apoptotic protein Bcl-2. Expression of Bcl-2 was examined immunohistochemically in human renal biopsy specimens comparing NP IGAN (n = 9) and P IGAN (n = 12). A standard four-layered immunoperoxidase based technique was used. Interstitial infiltrate, B and T cell expression was also measured. In adition, gene expression of Bcl-2 was measured in RNA extracted from human biopsy samples, by quantitative RT-PCR in NP IGAN (n = 10), P IGAN (n = 10) and compared to normal nephrectomy samples (n = 7). The immunohistochemistry revealed disease progression to be associated with interstitial B-cell infiltration ($p < 0.01$). Bcl-2 was expressed in glomeruli and in the interstitum and correlated strongly with T-cell infiltration ($p < 0.001$). Increased interstitial Bclk-2 expression correlated strongly with disease progression ($p < 0.02$). The results of RT-PCR were as follows (geometric mean with 95% CI):

	Normal	NP IGAN	P IGAN
Bcl-2 mRNA copies/ng RNA	39 (9–173)	146 (79–272)	281 (162–489)

Bcl-2 expression was increased in patients with IGAN (NP and P) versus controls ($p < 0.035$) and in P versus controls ($p < 0.05$) but no significant difference was seen between NP and P. These results suggest that Bcl-2 may play a role in both forms of the disease. In NP IGAN, glomerular Bcl-2 expression may inhibit apoptosis and thus allow low grade mesangial proliferatrion to persist. In P IGAN, Bcl-2 may inhibit apoptosis of infiltrating inflammatory cells and thus potentiate glomerulosclerosis, interstitial fibrosis and atrophy.

P135

UTERINE TISSUE-SPECIFIC INHIBITION OF INTERLEUKIN-10 PRODUCTION IN BABOONS, A CYTOKINE WITH 97% HOMOLOGY TO HUMANS

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The value of the baboon in predicting human reproductive immune IL-10 function is dependent on similarities in cytokine production, structure and function between humans and baboons. This study aims to functionally inhibit IL-10 production from baboon reproductive tissue and peripheral immune cells, *in vitro* using a human IL-10 monoclonal antibody (mAb), as well as sequence the baboon IL-10 gene and determine its homology to humans. Endometrial tissue (via dilatation and curettage) and PBMCs (via whole blood) were taken from baboons (n = 6) under ketamine anaesthesia (5 mg/kg i.m.) and incubated in RPMI culture medium (CM). Cells (1×10^5) and tissue were either unstimulated or stimulated with PHA (1/100) in the absence or presence of human anti-IL-10 mAb (20 µg/ml). Commercially available ELISA kits ImmunoKontact™ were used to determine IL-10 concentrations in the CM. WBCs from endometrial cultures were separated and counted using a Coulter-counter with endometrial IL-10 production expressed as pg/ml/million WBCs. Statistical difference between all groups was tested using the Kruskal-Wallis test. The RT-PCR product from RNA extracted from female baboon liver (using Trizol reagent) was purified and sequenced using Big Dye Terminator v2 kit on an ABI 3700 automated sequencer. IL-10 production from cultured endometrial tissue was almost completely blocked with human IL-10 mAb under stimulated conditions (42.5 ± 15.7 to 3.4 ± 2.5 pg/ml/10^6 cells, $P < 0.05$). IL-10 production from cultured and stimulated PBMCs was reduced by 26% with the IL-10 mAb. There is a 95% amino acid and 97% nucleotide sequence homology between humans and that predicted for baboons. In the current study we have demonstrated that production of IL-10 by baboon reproductive tissue is susceptible to inhibition with human mAb and identified a high sequence and functional similarity between human and baboon IL-10.

P134

PROTEIN STIMULATION INDUCED CYTOKINE AND APOPTOTIC SIGNALS IN PROXIMAL TUBULE CELL (PTE)

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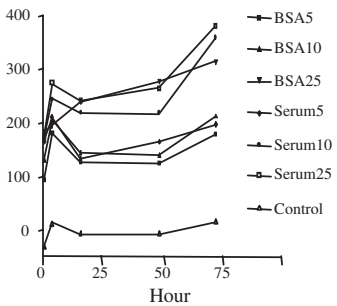
Protein loading of PTEs results in secretion of cytokine signals and apoptosis. Primary cultures of human PTE were stimulated by bovine serum albumin (BSA) and human serum proteins separated by gel filtration column chromatography into the 30–100 kDa fraction. Stimulant protein at concentrations of 5, 10, and 25 mg/ml at the apical surface induced similar time- and dose-dependent basolateral release of TFG-b1 and RANTES measured by ELISA (see figures). Apoptosis was detected by the immunofluorescent M30 antibody, that targets the caspase cleavage product of cytokeratin 18, at 72 hours post-stimulation. The percentage of apoptotic cells increased with increasing concentrations of stimulant protein. These results show that apoptosis, like release of cytokine signals, is dependent on the concentration of stimulant protein.



P136

ACE INHIBITION PRESERVES GLOMERULAR NEPHRIN EXPRESSION IN EXPERIMENTAL DIABETIC NEPHROPATHY

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Background and Aims: Mutations in the gene coding for the podocyte slit pore membrane protein, nephrin, are responsible for the Finnish-type congenital nephrotic syndrome. The present study sought to: (1) determine whether nephrin expression may also be altered in experimental diabetes, an acquired renal disease characterized by progressive proteinuria and (2) assess whether anti-proteinuric therapy with ACE inhibition modulated nephrin expression in the diabetic context. **Materials and Methods**: Male Sprague Dawley (SD) rats, 8 weeks old, were randomised to control (C) and diabetic (D) (Streptozotocin [STZ]-treated) groups, with and without perindopril treatment. Rats were sacrificed at 6 months after STZ injection. Kidneys were removed and the glomeruli were isolated by serial sieving. Real-time PCR analysis of cDNA (reverse-transcribed from mRNA) from the glomeruli of these rats was performed using a TaqMan® probe and primers for rat nephrin and 18S rRNA, and a GeneAmp 5700 Sequence Detection System (PE Biosystems). **Results**: STZ-treated rats were all diabetic (glucose > 15 mmol/l) and exhibited albuminuria at 6 months. Compared with the control animals, glomerular nephrin gene expression was significantly reduced in diabetic rats (see Table). In contrast, in perindopril-treated diabetic rats, glomerular nephrin gene expression was similar to control animals, and significantly greater than in untreated diabetic rats.

Group	Number/group	Nephrin : 18S mRNA (AU)
Control	9	1.04 ± 0.09
Diabetic	12	0.56 ± 0.11*
Diabetic + Perindopril	8	1.15 ± 0.24†

Data as mean ± SEM. * $p < 0.01$ vs Control group, † $p < 0.05$ vs Diabetic group.

Conclusions: These findings indicate that: (1) experimental diabetic nephropathy is associated with reduced glomerular nephrin expression and (2) normalisation of nephrin gene expression by ACE inhibition may contribute to the anti-proteinuric and renoprotective effects of these agents.

P136A

MACROPHAGE ACCUMULATION IN PROGRESSIVE DIABETIC NEPHROPATHY IN HUMANS

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Diabetic nephropathy is a major global health problem. Progression may be predicated by proteinuria, tubulointerstitial fibrosis and myofibroblast accumulation. Mechanisms of progression are unknown, however animal studies suggest a role for macrophages. We studied macrophage accumulation and clinical course of patients with diabetic nephropathy.

Methods: Retrospective study of baseline histology and 5 year clinical course. Twenty consecutive patients biopsied between 1990–95 with a histological and clinical diagnosis of diabetic nephropathy were studied. Macrophage accumulation in renal biopsy tissue from the 20 subjects and 5 normal controls was quantified by staining with KP-1 (antiCD68). Known predictors of progression (proteinuria, tubulointerstitial damage, myofibroblast accumulation) were also assessed at time of biopsy. Rate of progression was assessed by reciprocal of serum creatinine.

Results: Accumulation of macrophages was apparent in glomeruli (2.8 ± 0.7/gcs versus 1.0 ± 0.2 for normals, $p = NS$) and interstitium ($296.9 \pm 63.3/mm^2$ versus $19.0 \pm 1.3/mm^2$ for normals, $p = 0.002$). Glomerular macrophage number correlated with serum creatinine at time of biopsy ($r = 0.548$, $p = 0.012$) but not with progression. Interstitial macrophage accumulation correlated with serum creatinine ($r = 0.65$, $p = 0.002$), proteinuria ($r = 0.78$, $p < 0.0001$), interstitial fibrosis ($r = 0.77$, $p < 0.0001$) & inversely with slope of 1/serum creatinine ($r = -0.53$, $p = 0.02$). Proteinuria & myofibroblasts were also predictive of progression.

Conclusion: Macrophages accumulate within glomeruli and the interstitium in diabetic nephropathy and the intensity of the interstitial infiltrate is proportional to the rate of progression. Interstitital accumulation correlates with known markers of progressive nephropathy. This human data supports animal studies which suggest a pathogenic role for the macrophage in diabetic nephropathy.

P137

NUTRITIONAL STATUS AND VASCULAR RISK FACTORS IN PRE-DIALYSIS CHRONIC RENAL INSUFFICIENCY

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Atherosclerotic vascular disease is prevalent in patients who commence dialysis and significant relationships have been demonstrated between the presence of vascular disease, malnutrition and markers of chronic inflammation. The aim of this study was to examine the relationship between renal function, nutritional status and vascular risk factors in predialysis chronic renal insufficiency (CRI).

59 consecutive patients (age 68 ± 2 years, 68% male) with CRI (creatinine 292 ± 22 umol/L, calculated GFR 27.4 ± 1.7 ml/min) underwent a structured history and examination for determination of vascular risk factors and nutritional status (Subjective Global Assessment). Fasting plasma C-reactive protein (CRP), homocysteine, lipids, glucose and albumin were measured.

31% of patients were malnourished. Poor nutritional status was inversely correlated with creatinine clearance ($r^2 = 0.12$, $p = 0.006$) and positively correlated with CRP ($r^2 = 0.1$, $p = 0.04$). There was a negative correlation between creatinine clearance and plasma homocysteine ($r^2 = 0.34$, $p < 0.001$), but no relationship between renal function and lipids, glucose or serum albumin.

These data suggest an association between decreased renal function, poor nutritional status and chronic inflammation in CRI. Decreased renal function is also associated with hyperhomocysteinemia. The prognostic significance of these associations remains to be determined in prospective studies.

P138

ATHEROSCLEROTIC RISK FACTORS AND VASCULAR DISEASE IN CHRONIC RENAL IMPAIRMENT

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The prevalence of vascular disease in the chronic renal impairment (CRI) prior to the institution of dialysis has been poorly characterised. The aim of this study was to determine the prevalence of risk factors for atherosclerosis and the presence of vascular disease in a cohort of patients with CRI.

A retrospective chart review of 150 consecutive CRI patients referred to the outpatients department of a metropolitan tertiary referral centre was performed. The cause of renal impairment, the presence of vascular risk factors and the presence of vascular risk factors and the presence of vascular disease (determined clinically) was noted. Creatinine clearance was estimated by the Cockcroft Gault formula. Results are expressed as mean, 95% confidence internal).

Patient population was elderly mean age 69 with causes of renal impairment as follows: 42.7% ischemic nephrosclerosis (IN), 18.7% diabetic nephropathy (DN), 17.3% chronic glomerulonephritis (GN) and 21.3% other causes (ON). In the total group there was an 89% prevalence of hypertension, 70% prevalence of dyslipidemia, 32% prevalence of diabetes and 37% previous smokers. There was a significantly higher number of risk factors in patients with DN (3.1, 2.9–3.4) and IN (2.3, 2.0–2.5) than ON and GN (1.7, 1.4–2.0). Vascular disease was present in 54% of the total population. Patients with IN had a higher number of vascular territories involved (1.2, 0.9–1.4) than patients with DN (1.0, 0.6–1.4), GN (0.5, 0.2–0.7) or ON (0.5, 0.2–0.7). In multiple regression there was a significant relationship between patient age and vascular disease ($r^2 = 0.08$, $p = 0.03$) and a number of risk factors and vascular disease ($r^2 = 0.02$, $p = 0.001$) but no relationship between creatinine clearance and vascular disease.

These data demonstrate that atherosclerotic risk factors and vascular disease are prevalent in patients with CRI. The prevalence of vascular disease appears to be principally related to age and classical vascular risk factors rather than renal impairment per se.

P139

ALBUMINURIA AS A CARDIOVASCULAR RISK FACTOR: CLUSTERING WITH CRP AND CLASSIC RISK FACTORS IN A REMOTE ABORIGINAL COMMUNITY

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Rates of renal and cardiovascular disease are high in Aboriginal communities in Northern Australia. This environment is also marked by poor housing, nutrition and repeated bacterial infections. Inflammatory & nutritional factors may also play a role in renal and cardiovascular disease in this environment.

In one community we performed a survey ($n = 237$, 58% adult population) of renal disease (including spot urine albumin/creatinine ratio—ACR), carotid intima-media thickness (IMT, a surrogate marker of cardiovascular risk) and putative atherosclerotic risk markers (including fibrinogen, C-reactive protein (CRP), CMV, Chlamydia pneumoniae (CP) & H. pylori (HP) serology). ACR classed as normal (< 3.4 g/mol), micro- (MA-ACR 3.4–33.9) & overt- albuminuria (OA-ACR > 34)

Albuminuria was present at high rates overall (13% OA, 31%MA). ACR in the OA range was related to higher CRP (OR 2.9 [1.1–8.1] for top quartile of CRP), fibrinogen (OR 4.7 [1.7–13]) and inversely to serum albumin as well as diabetes (OR 6.0 [2.4–15]) and hypertension (OR 5.5 [2.0–14] for DBP > 90 mmHg). ACR was also related continuously to CMV IgG titre ($r = 0.39$, $p < 0.01$), independent of associations of ACR with CRP or total IgG. There was no association of ACR with HP or CP serology. Hypertension, obesity, hyperglycaemia; low HDL cholesterol clustered together ($p < 0.001$). OA was associated with the occurrence of 3 or 4 of these factors (OR 4.3 [1.6–11]). CRP was 1.6 [1.3–2.2] times higher in this cluster of risk factors; CMV IgG (but not total IgG) also associated significantly with this cluster.

OA was also associated with IMT (OR 4.3 [1.6–11] for upper quartile of IMT), independent of associations of IMT with HbA1c, BP, CRP, fibrinogen, total IgG and CMV IgG, and also independent of the 3RF cluster (also associated with IMT).

These mark a complex web of risk markers for renal and cardiovascular disease extending beyond the tradition BP, lipids, smoking and diabetes to include albuminuria, infective and inflammatory indices.

P140

MACROPHAGE-COLONY STIMULATING FACTOR (M-CSF): A RISK FACTOR FOR ATHEROSCLEROSIS IN URAEMIA?

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There is evidence for an inflammatory basis for atherosclerotic disease. An early event in plaque formation is macrophage infiltration and proliferation. M-CSF is a key regulator of macrophage function. The aim of the present study was to evaluate the relative contribution of plasma M-CSF concentration to the severity of atherosclerosis in 154 uraemic patients (age 56.2 ± 1.2 years, 59% males, 60% vascular disease) and 54 matched controls (age 55.0 ± 2.1 years, 63% males, 70% vascular disease). A medical history and risk factor status, (lipid, homocysteine, high sensitivity C-reactive protein (hsCRP) and M-CSF levels), were evaluated in each patient. Vascular structure and function were respectively assessed by carotid intima-media thickness (IMT) and brachial artery reactivity (BAR). M-CSF levels were elevated in uraemic patients (2514 ± 125 pg/ml vs 736 ± 51 pg/ml, $p < 0.001$) and were independently associated with renal failure ($p < 0.001$), CRP ($p = 0.002$), smoking status ($p = 0.001$) and diabetic status ($p < 0.001$). Both carotid IMT and BAR were comparable between the uraemic and control groups (0.68 ± 0.13 mm vs 0.65 ± 0.12 mm and 11.5 ± 0.6% vs 11.3 ± 1.3%, respectively, p = NS). On multivariate analysis using a general linear model, IMT was significantly associated with age ($p < 0.001$) and smoking status ($p = 0.003$), whilst BAR was associated with age alone ($p < 0.001$). M-CSF levels were not independently predictive of either IMT ($p = 0.17$) or BAR ($p = 0.34$). In conclusion, M-CSF levels are markedly elevated in uraemic individuals and correlate with several potent risk factors for cardiovascular disease. However, there is no independent association between M-CSF concentrations and atherosclerotic burden.

P141

TNFA, TGFB AND IL-10 GENE POLYMORPHISMS IN THE PROGRESSION OF IGA NEPHROPATHY (IgAN)

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Previous studies have demonstrated an association of the −308 TNF promoter polymorphism (TNF2) and progression in certain inflammatory disorders. TNF2 and IL-10*A *negativity* have been associated with renal transplant rejection whereas $Arg^{25} \rightarrow$ Pro TGF 1 has been associated with lung allograft fibrosis. These alleles are associated with cytokine up-regulation. To examine their role in the progression of IgAN, the frequency of these polymorphisms in 23 patients with progressive disease (≥ 25% decline in creatinine clearance to below the normal range or ESRF) was compared to 61 patients with normal/stable renal function. For TNF2 genotyping, PCR primers were constructed to incorporate the polymorphic site into a *Nco*1 restriction site in TNF1 but not TNF2. For $Arg^{25} \rightarrow$ Pro TGF 1 and IL-10*A polymorphisms, the ARMS-PCR technique was used. When corrected for age at biopsy and gender (both associated with progression; $p = 0.004$ and $p = 0.02$ respectively), the frequency of TNF2 and $Arg^{25} \rightarrow$ Pro TGF 1 was *not* significantly increased among "progressors" compared with "non-progressors". There was a trend toward IL-10*A *negativity* amongst "progressors". Hence, these alleles are unlikely genetic markers for progression in IgAN although other polymorphisms within these genes remain to be analysed.

	"Progressors"	"Non-progressors"	p
Male	36.25%	63.75%	0.02
Female	12.5%	87.5%	0.02
TNF2	42.3%	19.6%	0.20
$Arg^{25} \rightarrow$ Pro TGF 1	25.0%	24.3%	0.67
IL-10*A *negative*	42.11%	21.54%	0.06

P142

RENAL PARENCHYMAL RESISTANCE INDEX IN TYPE 2 DIABETES

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Traditionally, microvascular disease resulting in a glomerulopathy and an increase in albumin excretion rate (AER) is believed to be the only significant mechanism by which diabetic nephropathy develops. A possible contribution from intra-renal macrovascular processes to the pathogenesis of renal failure in type 2 diabetes is still not well defined. The renal parenchymal resistance index (PRI) is a measure of arterial impedance which is not influenced by glomerular disease [1]. We investigated whether there was a relationship between PRI, glomerular filtration rates (Cockroft-Gault, CG-GFR) and albumin excretion rates (AER) in 66 patients with type 2 diabetes recruited from our endocrinology clinics (mean HbA1c ≥ 7.9%). The mean PRI was 0.75 ± 0.01 (normal ≤ 0.7 [2]). PRI was significantly higher in diabetics with macroalbuminuria, i.e., AER > 200 μg/min (PRI = 0.81 ± 0.02, n = 11, $p < 0.02$) compared to those with either normoalbuminuria (PRI = 0.74 ± 0.01, n = 29) or microalbuminuria, i.e., AER = 20–200 μg/min (PRI = 0.73 ± 0.02, n = 26). Patients with macroalbuminuria also had a lower CG-GFR (52 ± 3 ml/min, $p < 0.01$) compared to those with normoalbuminuria (69 ± 3 ml/min) or microalbuminuria (73 ± 5 ml/min). PRI was significantly correlated with CG-GFR ($r = -0.55$, $p < 0.001$) and to a lesser extent with log AER ($r = +0.31$, $p < 0.05$). These findings suggest that intra-renal macrovascular pathology may play a role in the development of nephropathy in type 2 diabetes.

[1] Pratt *et al.* (1990), AJR, 154, 1223–1227. [2] Pratt *et al.* (1994), Radiology, 190, 343–346.

P143

RELEVANCE OF IONISED CALCIUM MEASUREMENT IN ASSESSMENT OF AND PRESCRIPTION FOR RENAL OSTEO-DYSTROPHY

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Free ionised calcium (iCa) constitutes approximately 50% of total serum calcium (tCa) and is the only fraction with biological activity and subject to homeostatic control. In this study, we examined the relationships between iCa, tCa and albumin-corrected calcium (cCa), phosphate (P), albumin and parathyroid hormone (PTH) status in 52 stable chronic haemodialysis patients. Overall, iCa was highly correlated with tCa ($r = 0.617$, $P < 0.0001$) and cCa ($r = 0.68$; $p < 0.0001$). In multiple regression analysis, iCa correlated with tCa ($p < 0.0001$) and serum albumin ($p = 0.028$) but not with bicarbonate or PTH ($P > 0.05$). Replacing tCa with cCa in the model eliminated the dependence on serum albumin ($p = 0.628$). PTH was predicted mainly by P ($p = 0.006$) and thus by Ca-P product ($p < 0.010$ for both iCa-P and tCa-P) and also serum albumin ($p = 0.021$ and 0.033 repectively) but not by bicarbonate or Mg. Stratified for serum albumin, with a level > 39 g/L, iCa correlated strongly with cCa ($r = 0.88$; $p < 0.0001$; n = 17), but the correlation was weaker at albumin levels 36–39 g/L ($r = 0.47$; $p = 0.027$; n = 22) and totally lost when albumin fell below 36 g/L ($r = 0.476$; $p = 0.139$; n = 11). These results suggest that tCa and cCa may be misleading when serum albumin is low, and that iCa should then be used to correctly assess calcium status and guide appropriate drug prescription. They also demonstrate the overriding importance of phosphate control.

P144

RELATIONSHIP BETWEEN CREATININE CLEARANCE AND HOMOCYSTEINE IN PREDIALYTIC STAGE CHRONIC RENAL FAILURE PATIENTS

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In order to determine the relationship between creatinine clearance (CCT) and homocysteine levels in predialytic chronic renal failure patients we calculated CCT using Cockroff-Gault formula for men and women and fasting total plasma homocysteine levels in those patients at out-patients Clinic Sanglah General Hospital Denpasar Bali.

Twenty six patients age 58.75 ± 12.71 years wih body weight 55.02 ± 8.34 kg, serum creatinine levels 4.21 ± 2.09 mg/dL, and CCT 18.69 ± 12.11 ml/mnt. Using 15 ml/mnt as cut-off value of CCT, 14 patients had CCT less than 15 ml/mnt and 12 patients had CCT 15 ml/mnt or more. There was significant higher levels of homocysteine in patients who had CCT less than 15 ml/mnt than those who had 15 ml/mnt or more (25.02 ± 9.89 vs 17.00 ± 5.06 mg/dl, $p = 0.01$). Using bivariate Spearman correlation test, there was a significant negative correlation between CCT and homocysteine levels ($r = -0.37$, $p = 0.05$) was found. Using linear regression test, the regression equation was (homocysteine) = 26.46–0.27 (CCT). This results suggest that in predialytic stage of chronic renal failure hyperhomocysteinemia which is considered as cardiovascular risk factor is related to the reduced renal function.

P145

ANTI-ERYTHROPOEITIN ANTIBODIES AS A CAUSE OF PURE RED CELL APLASIA IN END STAGE RENAL FAILURE AFTER RECOMBINANT HUMAN ERYTHROPOEITIN THERAPY (rhuEPO)

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There are few reports in the literature describing anti-erythropoeitin antibodies (antiEPO) as a cause of refractory anaemia and fewer describing this in association with pure red cell aplasia (PRA). We describe a 55 year old male of Chinese-Caucasian descent with end stage renal failure secondary to hypertensive nephrosclerosis who develops a rapidly progressive and refractory anaemia despite previously adequate doses of rhuEPO (alpha) and iron supplementation (target haematocrit 32–36%). The patient had been on haemodialysis for 10 months and rhuEPO therapy for 1 year. Investigations revealed no evidence of gastrointestinal bleeding, haemolysis, haemoglobinopathies, paraproteins or hypogammaglobulin-aemia and serum aluminium level was not elevated. Reticulocytes were undetectable and bone marrow examination results indicated a PRA. Investigations in search of a thymoma, acute parvovirus infection and human immunodeficiency virus were unrevealing. His background was notable for previous chronic active hepatitis B with e antigenaemia and high levels of circulating deoxyribonucleic acid (DNA) for which he underwent spontaneous seroconversion and sustained loss of hepatitis B DNA. AntiEPO antibodies were detected by immunoprecipitation. Nine months after withdrawal of rhuEPO, the patient remains transfusion dependent and has developed iron overload requiring chelation. Further quantitative assays of his antibody titre are in progress, as are studies of its effects on erythropoeisis in vitro and its relationship to endogenous EPO levels. Whether or not to proceed with renal transplantation remains a therapeutic dilemma. We conclude that the development of antiEPO antibodies is a rare cause of EPO-refractory anaemia, which may be characterised by red cell aplasia.

P146

SCREENING TECHNIQUES IN THE DUPLICATED REGION OF THE POLYCYSTIC KIDNEY DISEASE 1 (PKD1) GENE IN PKD1-LINKED AUSTRALIAN FAMILIES

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Adult polycystic kidney disease (ADPKD) is an autosomal dominant condition accounting for up to 10% of patients on dialysis. There are many extra-renal associations, some of which can be fatal. 85% of Caucasian ADPKD patients have a germline mutation within the PKD1 gene located on chromosome 16p13.3. It has been shown that a second somatic mutation may be required for cyst development. Using PKD-1 specific polymerase chain reaction (PCR) amplification, we identified 43 DNA germline sequence variations in the duplicated region of the PKD1 gene in 17 unrelated affected individuals. Twelve of the novel mutations identified were private to individual families, these mutations segregated with the disease phenotype and indicated a change in protein structure. On this basis, they were deemed to most likely be pathogenic. This indicates a mutation detection rate of approximately 70% was achieved in this study. The novel mutations found in this study, along with previously documented mutations, will help to identify the molecular genotype of asymptomatic affected family members. As the number of mutations in the gene increase, it may become possible to determine if genotype-phenotype correlations exist in ADPKD.

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SMOKING AND RENAL FAILURE IN POLYCYSTIC KIDNEY DISEASE (ADPKD)

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To determine the relationship between smoking and renal failure in ADPKD, data from a database on 156 ADPKD patients (50% male, 73% hypertensive) from Australia and Poland was examined. Smoking history by questionnaire categorised patients as non- ($n = 64$), ex- ($n = 52$) or current ($n = 40$) smokers. Smoking was quantified as < 20 years ($n = 62$) or > 20 years ($n = 30$), and cigarettes per day: < 20 ($n = 57$), > 20 ($n = 16$). Two end points were examined (1) chronic renal failure (CRF, s. creatinine ± 150 umol/L, $n = 67$) and (2) end-stage renal failure (ESRF, $n = 33$), using Chi-square, Cox-regression analysis (adjusted for age, BP, PKD genotype & gender) and Kaplan-Meier analysis.

A graded and statistically significant increasing risk of CRF and ESRF was found with smoking duration (non-smokers/< 20 yr/> 20 yr: CRF 34/36/63% $p = 0.01$; ESRF 17/20/40% $p = 0.04$). Current smokers had an increased adjusted risk of developing CRF (Odds Ratio 2.1, CI 0.8–5.8, $p = 0.08$) and ESRF (OR 3.4, CI 0.8–10.5, $p = 0.07$). No difference in number and size of renal cysts was seen in smokers vs non-smokers.

This study shows that smoking gives an independent 2–3 fold increased risk of renal disease progression in ADPKD, probably through interstitial & vascular mechanisms, rather than by affecting cystogenesis.

P148

SAFETY AND EFFICACY OF SIMVASTATIN IN HYPERCHOLESTEROLAEMIC PATIENTS UNDERGOING CHRONIC RENAL DIALYSIS

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Hypercholesterolaemia is present in about 50% of dialysis patients. The present study compared the efficacy and safety of simvastatin plus an optimised lipid-lowering dialysis diet with placebo plus diet in a randomised, double-blind trial. Stable subjects treated with haemodialysis (HD) or peritoneal dialysis (PD) with serum total cholesterol >3.5 mmol/L, LDL>3.0 mmol/L and triglyceride <6.8 mmol/L after a six week dietary treatment phase and an eight week diet plus placebo run-in phase, were enrolled in a 24 week double-blind treatment phase. Fifty seven subjects (16 males, 41 females, median age 63 years) were randomised 2:1 to diet plus simvastatin (5–20 mg daily) or diet plus placebo for 24 weeks. Simvastatin dose was doubled bi-monthly if non-HDL cholesterol was >3.5 mmol/L. Simvastatin was significantly more effective than placebo in reducing serum cholesterol concentrations. For HD, the median percentage changes for total cholesterol (simvastatin versus placebo) were −21.4% and −12.1% respectively (P=0.011); for LDL cholesterol, −33.0% and −8.8% (P=0.023), and for non-HDL cholesterol, −25.2% and −14.0% (P=0.008). For PD, changes for total cholesterol were −*T22.1% and −1.5% respectively (P=0.003); for LDL cholesterol, −36.4% and 0.0% (P=0.001), and for non-HDL cholesterol, −24.9% and −3.6% (P=0.002). For both dialysis modalities, changes in HDL-cholesterol, triglyceride and Lp(a) concentrations were not significant (simvastatin versus placebo, P>0.05). The incidence of adverse clinical and laboratory events were not increased in the simvastatin-treated patients. We conclude that simvastatin is a safe and effective treatment for hypercholesterolaemia in ESRF patients.

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P149

MANAGEMENT OF CHILDHOOD NEPHROTIC SYNDROME IN AUSTRALIA

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Introduction: Childhood nephrotic syndrome (NS) is a rare (1 per 100 000) but important disease with a risk of relapse (70%), infection (10%) and thrombosis (2%). A systematic review of randomised controlled trials has shown that daily prednisone for 4–6 weeks, followed by 6–7 months of a tapering dose, reduces the risk of relapse by 60% compared with shorter courses of corticosteroid therapy following the first episode of NS without increase in steroid toxicity. This study was undertaken to determine the treatment regimens of Australian children presenting with their first episode of NS.
Methods: NS has been listed on the APSU card since July 1998 and it will be listed until June 2001. Initial and one year follow up questionnaires are sent to all doctors who report a case.
Results: Between July 1998 and December 2000, 107 new cases of idiopathic NS in children aged 0.25 to 15 years were reported. 91% children were treated with daily prednisolone or prednisone at a dose of 60 mg/m2/day or 2 mg/kg/day; 70% children received one dose per day, 26% received two doses per day and 4% received three doses per day. In 40% children, it was planned to give corticosteroids daily for 2–3 weeks (4%) or until the child achieved remission (36%). In the remaining children, the planned duration of daily corticosteroids was 4 weeks (42%), 6 weeks (10%), 8 weeks (4%), and 12 weeks (4%).
Antibiotic prophylaxis was given to 65 children; of these 45 received penicillin. Of the 95 children aged 2 years or more, 26 received pneumococcal vaccination and 18 were nephrotic and receiving daily steroids at that time. Albumin and diuretics were given to 33 children; 5 received diuretics alone and 11 albumin alone. Thirty four children received aspirin.
Conclusions: While two-thirds of Australian children with NS received daily prednisone for 4 weeks or more, one-third received shorter periods of daily steroids thus increasing their risk for relapse. Better systems are required to inform clinicians about the existing trial evidence for the use of corticosteroids in nephrotic syndrome to assist them with their decision making.

P150

ACUTE GLOMERULONEPHRITIS AND LANZOPRAZOLE THERAPY: A POSSIBLE ASSOCIATION-1

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We report the unusual case of an elderly woman who developed cutaneous lesion two days after the start of lanzoprazole therapy and acutre glomeralonephritis in the following weeks despite stopping lanzoprazole treatment. A 73 year old Jehova witness, with no renal disease, was treted with lanzoprazole 15 mg/d for a symnptomatic gastro-oesophageal reflux. Past hisotry included a mechanical aortic valve replacement, with oral anticoagulation since then. Forty-eight hours after starting lanzoprazole, she developed asthenia, nausea and marginated purpuric macula on the right leg. She was afebrile and physical examination was otherwise non contributiroy. Laboratory data showed Hb 11.5 g/dl, WBC 11.3 $10^{\wedge}3/\mu L$ (81% neutrophils, 14% lymphocytes, eosinophils not reported) plt 324 $10^{\wedge}3/\mu L$ and normal serum cratinine (SCr). Lanzoprazole was discontinued and the skin lesions were treated topically. Three weeks later she was admitted to the hospital because of nausea, malaise, fatigue, lack of appetite, peripheral oedema and an episode of macroscopic haematuria. Laboratory examination showed impaired renal function (SCr 250 µmol/L, CrC1 15 ml/mim), glomerular erythrocyturia and significant proteinuria. Immunologic work-up excluded viral or auto-immune disease. Although renal biopsy could not be performed, the patient was treated with sterioids termprarely associated with orneprazole. Prednisone was started at a dose of 1 mg/kg/24 h *80 mg/d), was slowly tapered down and discontinued after 9 months. The improvement of renal function and urinary findings was slow. The use of orneprazole during therapy of the renal disease could have dampened the response to sterioids. This suggests a hypersensitivity reaction to lanzoprazole, which was maintained by orneprazole. This could well indicate a class effect.

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NO PAIN—NO HAEMATURIA SYNDROME

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Fibromuscular dysplasia (FMD) is an uncommon disease of renal arteries of uncertain aetiology. Although it most commonly presents with hypertension, it can also masquerade a number of diseases. We report two cases in which the patients with loin pain were initially diagnosed as having renal colic and later discovered to have renal infarction as a result of dissection into the renal artery—a well-described complication of FMD. The first case was a 59-year-old female with serum creatinine 0.08 mmol/L, who had bilateral loin pain which was managed as renal colic, and she presented three weeks later with hypertensive encephalopathy. An angiogram confirmed that she had FMD with dissection into a segmental artery resulting in infarction of the mid-region of the right kidney, and consequent accelerated hypertension. The second case was a 35-year-old male with serum creatinine 0.09 mmol/L, who was discovered to have a recent infarct involving the lower pole on the right. He had a scarred lower pole on the left suggestive of a past silent ischaemic event. A renal angiogram confirmed that he had FMD, with dissection into bilateral inferior segmental arteries, causing infarction of the lower renal poles—the right-side being the more recent event. One surprising aspect of the cases was that there was no haematuria, despite the presence of renal infarction. To our knowledge, there is only one previous report of a patient with FMD who had spontaneous renal artery dissection leading to renal infarction without any significant haematuria. The recommended first line therapy is aggressive blood pressure control with pharmacotherapy, followed by renal angioplasty or surgery in cases with suboptimal blood pressure control. Dissection, however, can lead to substantial loss of renal mass, as occurred in these two cases, and the role of angioplasty or surgery is unclear. The prognosis for retention of renal function is also unknown.

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THREE CASES OF ACUTE RENAL FAILURE SECONDARY TO INGESTION OF WILD MUSHROOMS

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Acute renal failure as a presentation of mushroom poisoning has been described in a number of counties but not previously in Australia. This report describes three cases of young men who developed acute renal failure following ingestion of wild mushrooms for hallucinogenic purposes. None of the men experienced hallucinations, suggesting the cause of the poisoning in these cases was species misidentification. In two of the cases no recovery of renal function occurred resulting in the need for long term dialysis. Renal biopsy in these two cases showed a pattern of tubulointerstitial nephritis with early development of interstitial fibrosis. The most recognized mushroom associated nephrotoxin is orellanine. However, the actual mushroom species and toxin involved in these cases is not known. Mushroom foragers and medical practitioners ought to be aware of the various potential toxicities that may arise from the ingestion of certain species of wild mushrooms. The most important of these, in the Australian context, is fatal hepatotoxicity from poisoning by *Amanita phalloides*.

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NEPHROCALCINOSIS IN TWO WOMEN WITH ANOREXIA NERVOSA AND RENAL IMPAIRMENT

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Two patients with anorexia nervosa were referred with chronic renal impairment. One patient had previous episodes of severe hypercalcaemia requiring a number of intensive care admissions. The hypercalcaemia was extensively investigated but no cause found. She also admitted to laxative abuse. The other patient had obsessive compulsive disorder as well as anorexia nervosa. Laxative use was denied but demonstrated by spot urine laxative screen. Chronic diarrhoea and chronic persistent hypokalaemia were a feature of this woman's presentation. Both patients had negative urine screens for diuretics. Renal biopsy in both cases revealed nephrocalcinosis with chronic damage. The aetiology of the nephrocalcinosis remains unclear, although one patient had had severe hypercalcaemia. Previously described renal disorders in patients with eating disorders include electrolyte disturbances, chronic tubulointerstitial nephritis, hypokalaemic nephropathy, and unexplained chronic renal failure. The pathogenesis of the nephrocalcinosis in our patients with anorexia nervosa warrants further elucidation.

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THE ROLE OF HIPPURAN RENOGRAPHY AS A DIAGNOSTIC TEST TO DETERMINE TRANSIENT ACUTE RENAL FAILURE (ARF) EVENTS IN NEPHROTIC SYNDROME (NS) PATIENTS

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Spontaneous transient acute renal failure (ARF) with symptoms of orthostatic hypotension, tachycardia, peripheral vasoconstriction, nephrotic crisis, and diarrhea, is an extremely rare complication in adult nephrotic syndrome (NS). A simple and safe without specific preparation diagnostic tool but highly accurate is needed to diagnose transient ARF in minimal change NS. The objective of this study is to evaluate an oliguric state in NS patients by using a hippuran renography as a diagnostic test. This is a descriptive observational study. The samples were taken based on their number on hospital administration, after screening for inclusion criteria, the eligible were enrolled in this study. Standard protocol examination for INS, such as clinical, and routine laboratory examination were done in this study. The I^{-131} Hippuran renography were done two times, first at the oligury phase, and secondly after the subjects had been treated (diuresis phase). Seventeen INS patients were enrolled in this study (M: 12, F: 5), mean age 24, 2 (8,1) years, mean systolic blood pressure 120,2 (16,1) mmHg, and diastolic blood pressure 82, 4 (10,4) mmHg, mean serum ureum concentration 55,4 (23,0) mg/dl, mean serum creatinine concentration 1,5 (0,5) mg/dl, massive proteinuria > 5 gr/day (very selective PST n = 4; selective PST n = 11; non selective PST n = 2), total protein serum of 4, 7 (1,0) gr/dl, albumin serum of 2, 2 (0,6) gr/dl, total cholesterol serum of 417, 9 (131,8) mg/dl. In all patients the renogram pattern show a normal initial phase, followed by a secretion phase with a Tmax 6, 83 (0,8) minutes and followed by a slow down curve in the excretion phase. This renogram gave a partial bilateral-obstructive pattern look-alike. While diuresis occurred these renogram pattern reversed to a normal picture. The Hippuran renography could also be used as an early diagnostic test in NS with transient ARF.

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IS BLOOD PRESSURE RELATED TO KIDNEY SHAPE AND SIZE?

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Objective: To evaluate the relationship between blood pressure (BP) and kidney shape and volume in a population with high rates of renal failure.

Background: The relationship between the kidney and hypertension is complex as the kidney is one of the main organs affected by high BP and yet the kidney itself can be the cause of hypertension. There is evidence that a decrease in the number of nephrons leads to an increase in risk of hypertension. The number of nephrons correlates with the physical dimensions of the kidney, which can be simply and reliably measured by ultrasound.

Methods: A cross sectional community based study was carried out in a remote coastal Aboriginal community. All participants had a renal ultrasound examination, nthropometric and BP measurements. Kidney length, width and depth were measured and kidney volume was calculated ($KV = KL \times KD \times KW \times 0.523$). The ratio of kidney length to kidney transverse diameter was used as a measure of kidney shape.

Results: There were 552 participants (280M; 272F) with a mean age 26.8 years (range 4.4–70.2 years). BP measurements were higher in men and those with higher BMI, in children and in adults. Both systolic and diastolic BP correlated inversely with kidney volume, in males and in females. When corrected for age, sex and BMI, systolic BP was still significantly inversely correlated ($p = 0.045$) with kidney volume, but the relationship with diastolic BP was no longer statistically significant. There is an increase of 3.4 mmHg of systolic BP of every 100 ml decrease in kidney volume (adjusted for age, sex, bmi). Kidney volume, age, sex, BMI together explain 41.6% of the variance in systolic blood pressure. Kidney shape did not correlate with BP.

Conclusion: BP correlated with kidney size, but not kidney shape in this population.

The systolic BP correlated significantly with kidney volume, independent of age, sex and current BMI. The smaller kidneys probably reflect kidneys with lower numbers of nephrons, which are more prone to renal insults, hypertension and renal disease in later life.

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NOVEL ERYTHROPOIESIS STIMULATING PROTEIN (DARBEPOIETIN ALPHA) CORRECTS ANAEMIA OF EARLY CHRONIC KIDNEY DISEASE (CKD) AT A REDUCED DOSE FREQUENCY COMPARED WITH RECOMBINANT HUMAN ERYTHROPOIETIN (rHuEPO)

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Darbepoetin alfa, is a novel erythropoiesis stimulating protein with an approximately 3-fold longer terminal half-life than rHuEPO. The safety and efficacy of darbepoetin alfa for treatment of anaemia in early CKD patients (pre-dialysis) not treated with rHuEPO within 12 weeks (rHuEPO naïve) were evaluated. Patients with baseline hemoglobin (Hb) values < 11.0 g/dL (n = 166) were randomised on a 3 : 1 ratio to 0.45 mcg/kg darbepoetin alfa once weekly (approximately equivalent to 90 IU/kg rHuEPO weekly based on peptide mass) or 50 IU/kg rHuEPO twice weekly (100 IU/kg total weekly dose), administered SC for 24 weeks. Dose adjustments were made as required to achieve a Hb response (defined as a Hb increase of ≥ 1.0 g/dL from baseline and a Hb concentration of ≥ 11.0 g/dL).

The mean Hb increase over the initial 4 weeks of treatment was 1.38 g/dL (95% CI 1.21–1.55) in patients receiving darbepoetin alfa and 1.40 g/dL (95% CI 1.07–1.72) in those receiving rHuEPO. A Hb response was achieved in 93% (95% CI 87%–97%) of patients in the darbepoetin alfa group and 92% (95% CI 78%–98%) in the rHuEPO group. The median time to achieve target Hb in both groups was 7 (range 3–25) weeks. At the time of the Hb response, median weekly dose was 0.46 mcg/kg (range 0.3–2.3) for darbepoetin alfa and 100 U/kg (range 0–175) for rHuEPO. The safety profile of darbepoetin alfa did not suggest any safety concerns, and was consistent with that expected for early CKD patients.

In conclusion, darbepoetin alfa, given SC once weekly at a starting dose of 0.45 mcg/kg, was safe and effective for correction of anaemia in rHuEPO-naive patients with early CKD.