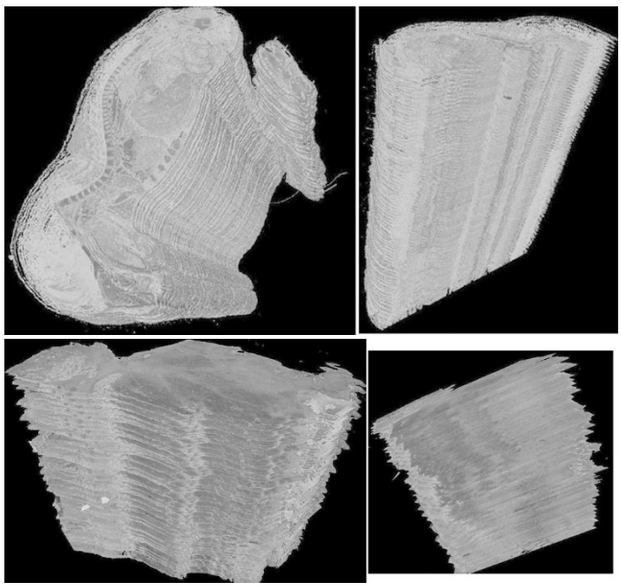

Conclusions: 3D reconstruction from multiple serial WSI sections can be used to generate impressive views of tissue. In the future this technique could be an important aspect of pathology analysis. However, we need to overcome many challenges to make this are routine process.

Kidney

1361 Segmental Sclerotic Lesions in Transplant Kidney: Insights in the Diagnostic Dilemma

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Background: Segmentally sclerotic glomeruli in the transplant (Tx) kidney may be due to recurrence of FSGS (Rec FSGS), or occur secondarily (Sec FSGS), e.g. related to hypertension, chronic allograft nephropathy, chronic Tx glomerulopathy or calcineurin inhibitor (CNI) toxicity. We examined nonimmune complex segmental sclerosing lesions in Tx, and classified based on clinical findings, time of diagnosis after Tx, light microscopic and EM findings.

Design: All Tx biopsies with segmental sclerosis and/or extensive foot process effacement (FPE) and negative IF from 1995 till 2006 were reviewed, excluding Tx glomerulopathy. All slides, reports, EM photomirographs, clinical history and follow-up were reviewed. Light microscopic (LM) findings were classified by the Colombia schema. Findings of Sec FSGS such as CNI toxicity, expanded lamina rara interna and limited FPE were assessed, and cases classified as Rec vs Sec FSGS.

Results: Fortytwo patients (29 male, 13 female) met entry criteria. Average age was 37±14 years (range 11 to 56). Twenty patients (48%) were African American and 13 (31%) were Caucasian. Twentythree (55%) had nephrotic proteinuria at the time of biopsy. Biopsy interval ranged from 4 days to 8 years after Tx. Twentythree (54%) case were classified as Rec FSGS, 15 (35%) as Sec FSGS and 4 (10%) as likely de novo FSGS. Ten (54%) Rec FSGS cases showed only extensive FPE, four (17%) cellular (CELL), four (17%) collapsing (COLL), and four (17%) not otherwise specified (NOS) lesions. In cases classified as likely Sec FSGS, NOS lesion was the most common morphologic variant, in 6 (40%), followed by 3 (20%) COLL, 2 (13%) CELL and 3 (20%) with FPE and other features of Sec FSGS. Rec FSGS was most common in early biopsies (85% of all FSGS cases in first 6 months). In contrast, 13 (65%) biopsies at > 2 years showed Sec FSGS. Nearly all patients, whether Rec or Sec FSGS, lost their kidney during the following months to years.

Conclusions: Early time of recurrence and extensive FPE were characteristic of Rec FSGS. NOS variant is more common in Sec FSGS, whereas extensive FPE alone is the most common finding in Rec FSGS. COLL, related to CNI toxicity, and CELL lesion can be seen in both Rec and Sec FSGS. We conclude that integrated analysis of LM, EM and clinical data help to differentiate varying etiologies of sclerotic lesions in the Tx.

1362 Coumadin Overdose Accompanied by Acute Tubular Injury: Pathological Findings in Renal Biopsies

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Background: Warfarin therapy has been associated with microscopic and gross hematuria. However, acute renal injury in these patients has been rarely described.

Design: Herein we report pathological findings in renal biopsies in patients with coumadin overdose and acute renal dysfunction.

Results: Seven renal biopsies from patients on coumadin therapy with gross hematuria were found in our database from 2004 till 2008. Mean age was 65.8±6.2 years. There were four males and three females. All patients had an increased INR (4.5±1.5 IU) and elevated serum creatinine (4.2±0.98 mg/dl)) at the time of biopsy, versus 1.3±0.41 mg/dl baseline levels. Four patients had proteinuria. Morphologically, all renal biopsies revealed red blood cells (RBC) in Bowman's space, RBC casts in tubules and acute tubular injury (ATI). Patients did not have history of extra-renal causes of ATI or underlying renal diseases resulting in gross hematuria. Hematuria was resolved after normalization of INR. Depletion of vitamin K in mice resulted in hematuria, but no tubular RBC casts and ATI, suggesting that an underlying renal condition is necessary for the development of RBC casts.

Conclusions: Occlusive RBC casts with acute tubular injury is a possible serious complication of a warfarin overdose and physicians should be aware of it.

1363 De Novo Membranous Glomerulonephritis Is Associated with C4d Depostion in Peritubular Capillaries and Basement Membrane Multilamination: Putative Variant of Chronic Humoral Rejection

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Background: The pathogenesis of de novo MGN in renal allografts is unknown. Animal studies support a role for non-MHC glomerular alloantigens. In a recent case report, the onset of de novo MGN correlated with development of anti-donor specific antibodies (DSA). Here we test the hypothesis that de novo MGN is a variant of chronic humoral rejection.

Design: All cases of de novo MGN and other forms of glomerular disease with available tissue were studied from transplant biopsies (1997-2008), C4d deposition (immunofluorescence) and electron microscopy. During this time C4d+ CHR was diagnosed on 8.4% of 738 transplant biopsies.

Results: De novo MGN had a higher frequency of C4d+ than the other de novo GN combined (p=0.017). Only de novo hepatitis C virus (HCV) GN was associated with C4d. Recurrent MGN and HCV were not statistically different from de novo. C4d+ de novo MGN commonly had mulitlamination of the GBM (4/4) and PTC BM (3/4), but this was not statistically greater than in C4d- de novo MGN (1/6 and 1/5 respectively; p>0.05). All de novo MGN patients tested for HLA-DSA were positive (3 class I, 1 also class II). One patient had three biopsies over 4 years all with de novo MGN, but C4d was present only in the last biopsy. One C4d- case had an earlier biopsy with focal C4d (10%).

Diagnosis	N Pts	C4d+ PTC	%C4d+
De novo MGN	16	6	38%
De novo FSGS	15	0	0%
De novo IgA	4	0	0%
De novo HCV	5	2	40%
De novo Misc GN	5	0	0%
Recurrent MGN	6	1	17%

Conclusions: C4d+ in PTC, the hallmark of CHR, is strongly associated with de novo MGN, but not other de novo GN except for that of HCV. The lack of a tight association argues that different antigens are the target of de novo MGN and CHR and fits with experimental data suggesting that non-MHC alloantigens may be the target. The association may be due to a propensity to form alloantibodies of any type or promotion of an immune response to glomerular antigens secondary to injury mediated by HLA antibodies. In any case, this study supports the concept that de novo MGN may be a variant of CHR.

1364 Challenges in the Diagnosis of Chronic Antibody Mediated Rejection Due to Loss of Peritubular Capillaries and Low Extent and Transient Nature of C4d Deposition

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Background: The diagnosis of acute antibody mediated rejection (AMR) is based on C4d deposition in >50% of peritubular capillaries (PTC). However, a loss of peritubular capillaries has been reported in late graft biopsies. Here we test whether the same criteria of C4d extent are appropriate for chronic AMR.

Design: All available cases of chronic humoral rejection (CHR) (1997-2008) were stained with two color immunohistochemistry for CD34 and C4d and compared with transplant glomerulopathy and no C4d (TG C4d-), chronic calcineurin inhibitor toxicity (CNIT), and normal transplant biopsies (NT). PTC density was quantitated visually and by morphometry (Aperio) and correlated with graft function and fibrosis (morphometry).

Results: Biopsies with CHR or TG C4d- had significantly reduced PTC density compared with NT (Table). Within the CHR group, the density of C4d+ PTC correlated with the overall capillary density (p=0.001). There was a wide spectrum of the % of PTC with (1-90%). Most cases (9) were Banff C4d2 (10-50%); a minority (7) were C4d3 (>50%). Most CHR cases had glomerular capillary wall C4d deposition (83%). The % fibrosis correlated with the Cr at the time of biopsy (p=0.04). C4d%+ and PTC density showed no statistically significant correlation with Cr at the time of the biopsy. PTC density was decreased with time post-transplant. Our data suggest that some TG C4d- cases are the sequelae C4d+ lesions, since 2/7 had had prior acute AMR. Furthermore one of the C4d+ CHR cases had a later biopsy that was TG C4d-. All of the CHR recipients tested had donor specific antibody (5 class I only, 6 class II only and 2 both), including the one with 1% C4d+ PTC and C4d+ glomeruli. Aperio microvascular counts were highly correlated with visual counts (r=0.928), although they were consistently higher.

			Capillary Density/mm2			CD34/mm2
Diagnosis	N	yrs post tx	CD34	C4d	%C4d+	p vs NT
CHR	17	9.2±6.1	228±108	109±113	44±33%	<0.03
TG C4d-	7	13.9±12.6	165±138			0.006
CNIT	6	5.2±3.2	243±185			n.s.
NT	7	0.4±0.7	319±188			

Conclusions: Diagnotic criteria of CHR should acknowledge the substantial decrease of capillary density and common occurrence of <50% C4d postive PTC. Glomerular C4d in the absence of glomerulonephritis may be a helpful criterion. C4d deposition can be transient in PTC while the TG persists.

1365 Alloantibody Levels and Capillary Endothelial Ultrastructural Features in Positive Crossmatch (+XM) Kidney Allografts

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Background: Glomerular and peritubular capillary endothelial cells show reactive changes in acute humoral rejection (AHR). We sought to determine whether antibody-mediated injury, as detected by electron microscopy (EM), correlates with alloantibody levels in +XM kidney allografts.

Design: A retrospective review was performed of consecutive +XM, ABO-compatible allografts during a two-year period. Protocol and indication biopsies with EM and serologic data from the time of biopsy were reviewed. EMs were reviewed without knowledge of antibody status and were categorized into groups, based on the presence (>25% of area) of reactive-appearing endothelial cells (REC) in glomerular (Glom) and peritubular capillaries (PTC). Corresponding donor-specific antibody (DSA) measurements by T and B cell flow crossmatch (FXM) and single-antigen beads for HLA class I and II (SAB-DSA-I, -II) were reported as mean fluorescence channel shift and as the mean fluorescence index (MFI), respectively. AHR was defined as C4d positivity with histologic evidence of acute tissue injury.

Results: Forty-one biopsies from 33 patients had electron microscopy performed and had serum DSA measurements near the time of biopsy. The mean time of biopsy post-transplant was 2.8 mos (range, 0-18). Most cases showed normal glomerular and PTC endothelial cells. Features of RECs included cell enlargement, loss of fenestrations, and cytoplasmic protrusions. The Glom and PTC REC groups showed higher T- and B-FXM channel shifts, higher MFI for SAB-DSA-I and -II, and a higher percentage of C4d positivity and AHR compared to the groups with normal endothelium.

EM Capillary Reactivity and Serum DSA Levels

Group	T-FXM	B-FXM	SAB-DSA I	SAB-DSA II	% C4d positive	% AHR
Glom-Normal (n=21)	111 (+/-140), n=16	160 (+/-138), n=16	1014 (+/-2090), n=13	3315 (+/-4289), n=13	5%	0%
Glom-REC (n=19)	236 (+/-148), n=12	356 (+/-133), n=12	5071 (+/-4748), n=14	6846 (+/-5504), n=14	70%	53%
PTC-Normal (n=25)	115 (+/-168), n=21	220 (+/-177), n=21	3080 (+/-4548), n=19	4218 (+/-4658), n=19	24%	8%
PTC-REC (n=16)	259 (+/-118), n=10	356 (+/-129), n=10	4502 (+/-3686), n=12	8032 (+/-5156), n=12	69%	50%

FXM and SAB expressed as mean (std dev)

Conclusions: The presence RECs in glomeruli and PTCs correlates with the serum level of DSA, and with C4d positivity and AHR. These findings may be useful in evaluating the effectiveness of new complement-inhibitory drugs that block complement at the level of the endothelium but do not affect serum DSA levels.

1366 Inter-Observer Agreement between Surgical and Renal Pathologist on Interpretation of Renal Donor Biopsies for Transplant Suitability

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Background: Microscopic evaluation of donor kidney biopsies is required to determine donor kidney suitability for transplant in selected cases. In our institution, frozen section interpretations are performed routinely by the surgical pathologist on call. Most specimens are wedge biopsies. After the tissue is snap-frozen, two hematoxylin and eosin-stained frozen sections are evaluated. To our knowledge, there are no data on inter-observer agreement between surgical pathologist (SP) and renal pathologist (RP) on interpretation of kidney donor biopsies.

Design: Retrospectively, 60 kidney donor wedge biopsies were retrieved from surgical pathology files in our institution and were reviewed by a renal pathologist (NG). The percent ratios (%) of glomeruli with global sclerosis, glomeruli and arterioles with fibrin thrombi, acute tubular necrosis (ATN), interstitial inflammation and fibrosis, the amount of arterial and arteriolar intimal fibrosis, and the presence/absence and extent of cortical necrosis were tabulated for each case. Results were compared with original reports signed out by ten different SP and kappa statistics were used to assess inter-observer agreement. P values less than 0.05 were considered statistically significant.

Results: Inter-observer agreement on interpretation of donor kidney biopsy by SP and RP was between "moderate" and "substantial" agreement with kappa values ranging between 0.42 and 0.71. The agreement was moderate for % glomeruli with thrombi (κ=0.48; p= 0.03), % of ATN (κ=0.46; p<0.001), % of interstitial fibrosis (κ=0.43; p=0.003) and the amount of arteriolar intimal fibrosis (κ=0.49; p=0.003). Substantial agreement was observed for % of globally sclerosed glomeruli (κ=0.70; p<0.001), % of interstitial inflammation (κ=0.63; p<0.001), and the amount of arterial intimal fibrosis (κ=0.72; p<0.001).

Conclusions: Our data show that SP as a group provide similar interpretations as RP on donor kidney biopsies for transplant suitability.

1367 Leukocyte Chemotactic Factor 2: A Novel Cause of Renal and Hepatic Amyloidosis Associated with Nephrotic Syndrome and Chronic Liver Disease

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Background: In this study, we describe a novel-type of amyloidosis caused by deposition of a chemokine, leukocyte chemotactic factor 2 (LECT2) which may account for the majority of unclassifiable amyloidosis seen in renal and liver biopsies.

Design: Eight cases of renal or hepatic amyloidosis, where amyloid type can not be determined after extensive pathological and clinical investigations, were studied. The amyloid typing was performed by immunohistochemistry for common types of amyloid (ATTR, SAA, AL) as well as LECT2 and by nano-flow liquid chromatography electrospray tandem mass spectrometry (MS/MS) following laser microdissection and proteolytic digestion of the amyloid deposits. The resulting MS/MS data was correlated to theoretical fragmentation patterns of tryptic peptide sequences from the Swissprot database using Scaffold algorithm. The identified proteins were examined for the presence or absence of amyloid related peptides. 90 cases of AL, ATTR and SAA amyloidosis and normal glomeruli from 10 different cases were studied as controls.

Results: Six of the patients were male, two female, the median age was 66 (range 41-76). Five cases presented with nephrotic syndrome. Two cases presented with chronic liver disease secondary to viral hepatitis and one case was an incidental finding in a liver biopsy performed during a cholecystectomy. In all cases, the amyloidosis appered to be localized to the kidney or the liver. None of the patient had family history or clinical findings supporting AL, SAA or ATTR amyloidosis. In each case, MS/MS analysis showed that one of the most abundant peptides was LECT2. No other amyloid associated peptides were present. In contrast, in the control cases involved by AL, SAA or ATTR and in normal renal tissue no LECT2 peptides were present. Immunohistochemical studies for AL, ATTR and SAA was negative whereast, in each case the amyloid deposits were strongly positive for LECT2 confirming MS/MS findings.

Conclusions: In this study, we described a novel-type of amyloidosis caused by deposition of LECT2. LECT2 amyloidosis typically presents with isolated renal involvement and nephrotic syndrome or liver involvement and chronic hepatitis. The clinical and pathological features of LECT2 amyloidosis closely mimic AL amyloidosis and should be considered in the differential diagnosis. The underlying cause for LECT2 amyloidosis remains unknown but an association with chronic inflammation is suggested in cases with liver involvement.

1368 Novel Subtraction Morphometry Technique for Assessing Fibrosis in Renal Biopsies

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Background: Quantitation of interstitial fibrosis has become of increasing importance for assessment of efficacy in clinical trials of drugs in transplantation and native kidney disease. Visual assessment is the standard practice using trichrome stained slides, but is poorly reproducible. The alternative, morphometry with special stains such as sirius red with polarization or anti-type III collagen IHC is cumbersome, requiring special processing. Therefore, we sought a new method that could be applied to routine renal biopsy stains.

Design: Trichrome stains all collagens, including basement membranes, which should not be included in measurements of interstitial fibrosis but are stained strongly by PAS. We reasoned that subtraction of the area stained by PAS from that stained blue by trichrome might be a robust measure of interstitial collagen. To test this, routine histology trichrome and PAS stained slides from 77 donor biopsies and 18 late transplant biopsies or nephrectomies were scanned with an Aperio ScanScope and analyzed using the ImageScope Positive Pixel Count algorithm with appropriate parameters that selected blue (trichrome) and pink (PAS). The % cortical interstitial fibrosis (T-P fibrosis) was calculated as (Trichrome area-PAS area)/total area and compared with a visual assessment.

Results: In 77 donor biopsies T-P fibrosis averaged 20±16 % (-6% to 64%). This reflects the finding that most donor biopsies had some fibrosis by visual scoring. Negative T-P fibrosis values (n=6) occurred when there was essentially no fibrosis and PAS area exceeded trichrome area. In late transplant samples, T-P fibrosis values were 31±21 % (4 to 75%). T-P fibrosis correlated moderately well with visual scores ($R = 0.41$, $P < 0.0001$). When analysis was performed at a different occasion on the same biopsy, essentially identical results were obtained. Subtraction removed the contribution of casts and sclerotic glomeruli and did not include tubular brush borders. The disparities between visual and morphometry seemed largely attributable to variability in staining intensity of trichrome and freeze artifact in donor biopsies.

Conclusions: The subtraction morphometry method for fibrosis provides the potential of routine histologic stains that are prepared on renal biopsies and widely available in clinical trials. The quantitative method is less subject to observer variability than visual assessment, but does require satisfactory tissue processing and staining.

1369 Pathology, Serology and Clinical Characteristics of 68 Patients with Chronic Humoral Renal Allograft Rejection (CHR)

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Background: CHR is a newly recognized category in the Banff classification system. We sought to define the spectrum of pathology, clinical features and pathogenetic insights by analysis of a large cohort of patients from one center.

Design: All renal transplant biopsies diagnosed with CHR based on Banff'05 criteria were identified in pathology and clinical databases (7/1993-7/2008). The pathology of the first C4d+ biopsies showing CHR were correlated with serology and clinical features.

Results: CHR was diagnosed in 68 recipients (48 males, 20 females; 7.8% of 997 biopsies), 7.7±5.2 yrs post-transplant (0.6-22), with a mean Cr of 4.6±2.8 mg/dl (1.3-11.9) and proteinuria >1 gm/24 hr (0.03-9.5) in 50%. All had C4d in peritubular capillaries (PTC), but 18% were focal. Five variants were recognized by combinations of transplant glomerulopathy (TG), transplant arteriopathy (TA) or interstitial fibrosis (IF). The commonest variant was TG+TA+IF (44%) followed by TG+IF (25%). However, TG negative forms were identified in 25% and an IF alone variant in 13%. CHR occurred together with acute humoral or cellular rejection in 15% and 16%, respectively. Donor specific antibodies (DSAs) were present in 89%; class II DSAs were associated with TG+ variants (P<0.0005). PTC multilamination present in 35/56 (64%) by EM did not

correlate with TG+. Glomerular and PTC mononuclear inflammation included CD68+, CD16+ (FcgR+) and Tbet+ cells with infrequent T cells. Prognosis was poor with 1 and 5 year graft survival 54% and 8%, respectively.

Variant	TG	IF	TA	C4d>50%	DSA+	DSA Specificity (%)	
N(%) of CHR						% I Only	% II Only
30(44%)	+	+	+	90%	90%	12%	75%
17(25%)	+	+	-	82%	91%	12%	50%
4(6%)	+	-	+	50%	100%	0%	100%
9(13%)	-	+	-	78%	100%	50%	25%
8(12%)	-	+	+	75%	67%	33%	0%
68(100%)				82%	89%(39/44)	18%	58%

Conclusions: CHR is common with heterogeneous pathology and combinations of TG, TA, IF and PTC multilamination, sometimes with superimposed acute humoral or cellular rejection. TG is strongly correlated with class II DSA. The usual criterion of >50% C4d deposition is insensitive for CHR (vs AHR). FcgR+ cells are abundant in capillaries and may be part of the pathogenesis. Diagnosis of CHR, which carries a poor prognosis, depends on recognition of the diverse pathologic manifestations.

1370 In-Vitro Mesangial Amyloid Formation Viewed by Scanning Electron Microscopy

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Background: Mesangial cells incubated with light chains (LCs) extracted and purified from the urine of patients with AL-amyloidosis (AL-Am) engage in the formation of amyloid. The process of amyloid formation involves internalization of the LCs into the mesangial cells and amyloid formation in the mature lysosomal compartment. How amyloid is extruded into the mesangial matrix remains unclear.

Design: Human mesangial cells were incubated with LCs obtained from the urine of patients with AL-amyloidosis for various periods of time up to 6 weeks. Samples were submitted for scanning electron microscopy at 1, 2, 5, 7, 10, 14, 17, 21, 28, and 42 days. After careful evaluation of each sample, representative photos were taken illustrating the process of amyloid formation in its various steps.

Results: Amyloid formation was confimed by using light microscopy in conjunction with Congo red and Thioflavin T stains. Mesangial cells were engaged in active amyloid formation in the samples obtained 24 hours after incubation with AL-LCs. Early fibril formation could be identified intra and extracellularly. The lysosomes were noted to be immediately adjacent to the cell membranes of the mesangial cells. As the time progressed, amyloid fibrils could be seen extruding from the surface of the mesangial cells. Once in the extracellular space, the fibrils appeared tangled and forming a mesh-like network.

Conclusions: This is the first time that in-vitro amyloid formation is monitored using scanning electron microscopy. Surface extracellular events associated with the formation of amyloid could be clearly observed. This study provides insights into the process of amyloid delivery to outside of the cells and confirms the non-branching fibrillary nature of amyloid and its peculiar fibril arrangement with random disposition.

1371 Apoptosis Is the Mechanism Associated with Mesangial Cell Deletion In Monoclonal Light Chain-Related Renal Diseases

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Background: The glomerular pathology in light chain deposition disease (LCDD) and AL (light chain-associated)-amyloidosis in its advanced stage is characterized by a loss of mesangial cells. The mechanisms involved in this process have not been completely elucidated. This study was conducted to evaluate the role of apoptosis in cell deletion in these diseases.

Design: Rat mesangial cells were incubated with light chains obtained and purified from the urine of patients with LCDD and AL-amyloidosis (10 ug/ml) for periods up to 6 weeks (n=6). Mesangial cell apoptosis was monitored using several techniques including light, electron and scanning electron microscopy, caspase 3 activation, annexin V expression on cell membranes, and propidium iodine to identify DNA changes associated with the apoptotic process. Curcumin was added to experimental conditions to evaluate if it had any effects on apoptosis.

Results: Apoptosis was significantly increased over controls when mesangial cells were incubated with both LCDD and AL-light chains. Apoptosis was more pronounced in mesangial cells treated with AL-light chains (approximately 2 times more) than with LCDD-light chains. The correlation between nuclear changes and cytosol / membranous alterations typically associated with apoptosis was excellent. Curcumin enhanced the apoptotic effect of both glomerulopathic light chains (almost 3 fold each). When rat mesenchymal stem cells were coincubated with rat mesangial cells and trated with glomerulopathic light chains, the apoptotic effect increased as well and the replacement of injured mesangial cells by mesenchymal stem cells occurred faster. Classical morphological findings were noted by routine and scanning EM in apoptotic mesangial cells.

Conclusions: Apoptosis represents the mechanism of cell deletion associated with the glomerular damage that occurs in LCDD and AL-amyloidosis. This explains the morphologic findings seen in glomeruli affected by these conditions, where in the advanced stage mesangial cells are few and extracellular matrix and amyloid fibrils respectively represent the main components of the expanded mesangium. If apoptosis is enhanced, the repair mechanism and replacement of injured mesangial cells by mesenchymal stem cells occurs faster.

1372 Genome-Wide mRNA Profiling To Define Shared Transcriptional Relationships and Renal Function in Multiple Renal Diseases

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Background: Morphologic examination of renal biopsies provides key information for the diagnosis and effective therapeutic management of renal disease. However, prognostic information has been limited. An international multicenter study was established and protocols developed to identify and validate molecular diagnostic markers and outcome predictors. Human renal biopsies have been obtained from 24 medical centers spanning 4 continents. More than 2000 biopsies have undergone manual microdissection of nephron segments and are available for gene expression analysis.

Design: Affymetrix HG-U133 based gene expression profiles from nine different renal diseases (focal and segmental glomerulosclerosis (FSGS), minimal change disease, membranous nephropathy (MGN), diabetic nephropathy, lupus nephritis (LN), IgA nephropathy, thin basement membrane disease, and controls) has allowed a network analysis approach to define relatedness of major human diseases on a molecular level. A bipartite network analysis, using the renal disease entities and mRNA expression regulation as nodes, creates an easily navigable graph demonstrating similarity of renal diseases on the transcriptomic level, revealing a shared transcriptional response among some diseases (FSGS, MGN, and LN).

Results: The bipartite network confirmed established progression factors of renal disease (e. g. apoptosis). But it also may implicate specific disease mechanism. In addition, expression profiles reflect the functional status irrespective of the cause of renal disease. Kidney tissues from six different diseases with a wide spectrum of creatinine clearances were studied. Using the ridge regression model for glomerular filtration rate (GFR) prediction, a marker panel of 40 mRNAs correlated with the GFR independent of histopathology ($r=0.78$, $p<0.001$). This panel performed equally well in a second series with three independent disease cohorts ($r=0.69$, $p<0.001$). and allowed the classification of chronic kidney disease (CKD) stage III-V versus stage I-II with a sensitivity of 0.73 and specificity of 0.82. In an independent cohort this mRNA marker panel was able to correctly predict the CKD stage 36 months after renal biopsy (sensitivity 0.77, specificity 0.75).

Conclusions: This demonstrates the feasibility of using cross-sectional mRNA markers to predict disease progression. S Martini and S Bhavnani contributed equally.

1373 CApillary DILatation Associated SegmentaL Sclerosis – CADILASS – a Separate Morphologic Form of Glomerular Sclerosis?

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Background: Focal and segmental glomerular sclerosis (FSGS) is the most frequent morphologic manifestation of glomerular injury seen in human biopsy material. While its etiology is diverse, the morphologic spectrum is less well defined. Nevertheless, correlation between different morphologic forms of FSGS and etiology has been attempted. Hitherto, we report a distinct form of FSGS associated with experimental nephron reduction, podocyte toxicity and hypertension and propose its recognition as a separate form of FSGS.

Design: Male rats (~250g, Harlan) underwent 1/2 or 3/4 surgical nephrectomy followed by a single dose of puromycin aminonucleoside (PAN) (75 mg/kg IP) 14 days after surgery. The animals were sacrificed at 4 wks post PAN, and kidney pathology was evaluated using H&E and PAS stains.

Results: Animals developed massive proteinuria, hypertension and FSGS. While less than 10% of glomeruli showed classic FSGS, many more glomeruli (20-30%, depending on type of procedure) showed only dilatation of individual glomerular capillaries clustered within a single or adjacent glomerular segment(s). There was a spectrum of lesions ranging from a segmental capillary dilatation to a frank sclerosis. The earliest lesions showed a pure segmental glomerular capillary widening not accompanied by mesangial matrix increase or loss. Cross-sections of widened capillaries were round and appeared first in the hilar region, either centrally or laterally. In more advanced lesions there was increase in mesangial matrix and capillary and capsular adhesions. While many capillaries became obliterated, occasional distended capillaries persisted into more advance stages of sclerosis, thus supporting the notion of a transition from capillary dilatation to FSGS. Both lesions were more pronounced in 3/4 nephrectomized animals. We, therefore, propose a term capillary dilatation associated segmental sclerosis -"CADILASS" to refer to such lesions. This particular form of FSGS appears to be a associated with podocyte toxicity, nephron reduction and hypertension.

Conclusions: We believe that, at least in this model, CADILASS is the earliest manifestation of glomerular injury leading to sclerosis and that this morphologic variant of FSGS has hitherto not been reported. If similar changes can occur in humans, their detection can be an early, possibly treatable, marker of a progression into glomerular sclerosis in patients with nephron reduction, such as transplant recipients.

1374 Non-Functioning Glomeruli as a Marker of Renal Damage

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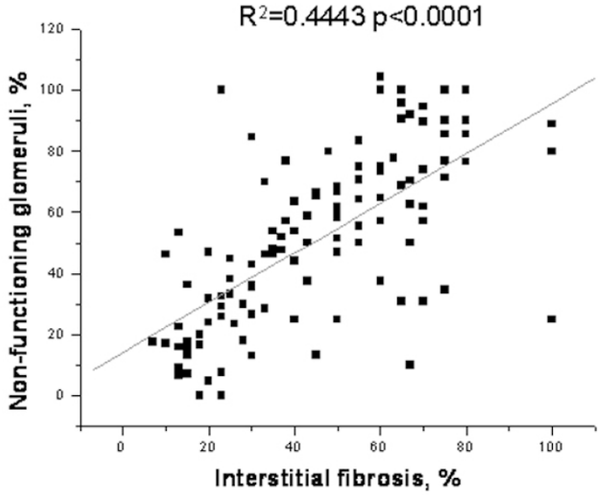
Background: It is well-established that the degree of interstitial fibrosis (IF) correlates better with renal function than the percentage of sclerotic/obsolescent glomeruli. However, non-functioning glomeruli include not only sclerotic glomeruli but also glomeruli with obvious periglomerular fibrosis (PF). Glomeruli with PF are frequently atubular or are connected to severely atrophic tubules and most likely do not contribute to glomerular filtration. The aim of our study was to examine the correlation between the number of non-functioning glomeruli (sclerotic and those with PF) with renal function and with the degree of interstitial fibrosis.

Design: A total of 114 native kidney biopsies taken from patients with chronic renal diseases were examined and assessed for the percentage of interstitial fibrosis in the renal

cortex and the number of non-functioning glomeruli. Findings were correlated with the serum creatinine level at the time of biopsy. Biopsies with any acute/active pathologic change and biopsies from patients with acute renal failure were not included in the study. The biopsies were then subdivided into two groups: 1) with diabetic nephropathy (n = 45) and 2) without diabetic nephropathy (n = 69).

Results: The percentage of sclerotic glomeruli did not correlate with IF (R sq.=0.0017, p = 0.6574), but the percentage of non-functioning glomeruli strongly correlated with the percentage of IF (R sq.=0.4443, p<0.0001).



The degree of IF positively correlated with serum creatinine levels (R sq.=0.4582, p<0.0001). In our study, the percentage of non-functioning glomeruli correlated better with serum creatinine (R sq.=0.2817, p<0.0001) than did the percentage of sclerotic glomeruli with serum creatinine (R sq.=0.2528, p<0.0001). This was particularly true in patients with diabetic nephropathy (R sq.=0.3973, p<0.0001).

Conclusions: The percentage of non-functional glomeruli (sclerotic glomeruli and glomeruli with PF) in a renal biopsy specimen provides a better estimate of chronic renal damage and renal function than the percentage of sclerotic glomeruli only. Therefore, the number of glomeruli with periglomerular fibrosis should be routinely included in the microscopic analysis of renal biopsies.

1375 Peritubular Capillary Pathology in Transplant Glomerulopathy

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Background: Transplant glomerulopathy (TG) is defined morphologically by glomerular basement membrane duplication in a renal allograft in the absence of immune deposits and evidence of thrombotic microangiopathy, and clinically by proteinuria and declining renal function. TG occurs in 10-20 % of grafts, shows association with alloantibodies (antibody mediated injury), cortical peritubular capillary C4d (PTCC4d) and basement membrane multilayering (PTCBMML), and when extensive, portends poor outcome. The etiology, pathogenesis, and features that affect prognosis remain unclear. Our aim was to examine pathological parameters that might further define TG and be useful in prognosis.

Design: Among 637 indication renal allograft biopsies between 2001 and 2005, TG was diagnosed in 58 (9%), 21 of whom had other secondary diagnoses, and were excluded. Histopathologic evaluation, immunohistochemistry for monocytes (CD68), PTC density (CD31), and angiogenesis (glomerular/tubulointerstitial VEGF and VEGFR), and outcome analysis (including creatinine clearance slopes) were performed on the 37 cases of pure TG.

Results: Mean patient age was 48±17 years, and mean graft age was 8.8±6 years at biopsy. PTCC4d was positive in 11(30%), and associated with severe PTCBMML (p=0.02). TG was associated with decreased PTC density (using CD31). Fourteen (38%) patients lost their grafts, and 3(8%) died with a functional graft. Worse graft-survival was associated with ≥ moderate interstitial fibrosis (p = 0.04), >5 PTC monocytes (p = 0.02), and was associated with a non-significant trend with ≥ moderate PTCBMML. Glomerular monocytes and PTCC4d were not associated with outcome. Creatinine clearance slopes became significantly more negative approximately 1 year before biopsy.

Conclusions: PTCC4d is seen in a subset of TG and correlates with PTCBMML. Tubulointerstitial disease and damage to the PTC were predictors of graft survival. Reduced PTC density suggests a role for PTC injury in the progression of TG. Based on creatinine clearance slopes, proteinuria occurs at a late stage in the evolution of TG, so that earlier (protocol) biopsies may be necessary to diagnosis TG if disease intervention becomes possible.

1376 In Silico Study of Glomerular Cyst Pathogenesis

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Background: Dilatation of Bowman's space >3 times normal constitutes a glomerular cyst (GC). Although linked to mechanical obstruction, drug toxicity or epithelial-mesenchymal interactions, the pathogenesis of GC is largely unknown. Recent molecular advances revealed underlying genetic mutations resulting in hereditary GC. However, a unifying pathogenetic mechanism is lacking. In this study, we applied bioinformatics in combination with the accumulated knowledge of cystoproteins in hereditary GC subtypes to identify common pathomechanisms.

Design: We reviewed 20 cases of GCK in our files and the entire published western literature of the last 150 years to a) determine the incidence of hereditary disease presenting with glomerular cysts and b) performed a comprehensive in-silico analysis using proprietary manually curated databases of human gene-protein interactions (Ingenuity Pathway Analysis and MetaCore Gene Expression and Pathway Analysis software) using shortest pathway functions and custom algorithms.

Results: Among our 20 cases, 15 were children and 5 were adults (age 30 weeks to 78 years). The majority were bilateral (n=17). Hereditary diseases (ARPKD, ADPKD, TSC, mitochondrial mutations) were present in 37.5% of the participants. The 242 reviewed publications showed 72% children; 12% hereditary (only 1% of cases confirmed as PKD through genetic testing) and less than 1% with documented uromodulin mutations or hepatocyte nuclear factor 1β (HNF-1β) mutations associated with diabetes of the young (MODY 5). High hits for molecular targets not previously associated with GC such as tumor suppressor genes and gene products localized in cilia were identified. The most important links were between: 1) HNF-1 β which acts upstream of PKHD1 (mutations causing ARPKD) and uromodulin; 2) ADPKD and contiguous gene syndromes are linked to TSC; 3) Uromodulin storage diseases that link GC to nephronophthisis and 4) NPH3 mutations (=renal-hepatic-pancreatic dysplasia) that provide common pathways with non-hereditary renal dysplasia.

Conclusions: While exact molecular targets for GC remain to be charted, these results suggest convergence of known cystogenesis genes into a common pathway of glomerulocystogenesis. Interactions are at multiple levels involving DNA, gene, and protein. This mechanism appears similar to the compelling new concept of ciliopathies encompassing kidney cystogenesis due to mutated gene products localized to primary cilia.

1377 Type I Interferons: Regulators of Inflammatory T Helper Cell Responses in SLE

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Background: Type I Interferon (IFN), α and β, are well recognized anti-viral agents that induce potent inflammatory T helper cell 1 (Th1) responses. Their effects on Th cell activity however, can be contrasting. As observed in multiple sclerosis patients, the beneficial effect of IFN-β therapy is partly due to the suppression of the Th1 type autoimmunity. In Systemic Lupus Erythematosus (SLE), the triggering of plasmacytoid dendritic cell (pDC) toll-like receptors (TLRs) by circulating anti-nucleic acid-containing autoimmune complexes stimulates pDCs to secrete high levels of type I IFNs. Study of both human and murine lupus disease strongly implicates type I IFNs as key disease effectors. However, while inflammatory, interferon (IFN)-γ-secreting Th1 cells are implicated in mediating serious forms of SLE, including nephritis and CNS lupus, the role of pDC-derived type I IFNs in regulating the function of these inflammatory Th cells in SLE is unknown.

Design: To investigate how type I IFNs regulate Th cell autoimmune inflammatory responses in SLE, we established an in vitro model of human peripheral blood mononuclear cell (PBMC) stimulation to mimic the effects of continuous type I IFN exposure.

Results: Our results indicate a subset of SLE patient sera that, when compared to normal donor sera, inhibits IFN-γ secretion by superantigen-stimulated healthy donor PBMC in a type I IFN-dependent manner. This effect positively correlates with PBMC secretion of the Th cell inflammatory cytokines, lymphotoxin (LT) and interleukin (IL)-17, while negatively correlating with IL-10 secretion. Remarkably, the ability of SLE patient serum to inhibit IFN-γ secretion in a type I IFN dependent manner correlates with positive serum titers of Sm and RNP, two anti-nuclear antibodies (ANAs) that occur in some SLE patients.

Conclusions: Our findings suggest that in SLE patients with significant serum type I IFN activity, type I IFN blockade may exacerbate Th cell-mediated autoimmune inflammation by inhibiting inflammatory cytokine-secreting autoreactive Th cells. Moreover, they suggest that specific ANA profiles can potentially be used as a biomarker to identify patients in which such a negative outcome is likely to occur.

1378 Type 2 Diabetic Patients with Greater Urinary Albumin Excretion and Less Severe Glomerulopathy

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Background: Proteinuria is commonly known as a marker of severe glomerulopathy in diabetic nephropathy (DN). While most renal structural-functional relationship (RSFR) studies of DN have been done in type 1 diabetes (T1DM), SFR in type 2 diabetes (T2DM) is not thoroughly understood.

Design: RSFR was studied in 161(M/F=60/101) T1DM and 133 (M/F=88/45) T2DM patients with a wide range of urinary albumin excretion rate (AER) and glomerular filtration rate (GFR). Mesangial fractional volume [Vv(Mes/glom)], glomerular basement membrane (GBM) width and peripheral GBM surface density [Sv(PGBM/glom)] were estimated in research biopsies using electron microscopy stereology.

Results: AER and GFR values were not statistically different between T1DM and T2DM patients. Multiple regression analysis in T1DM patients with Vv(Mes/glom), GBM with and Sv(PGBM/glom) as predictors and AER as dependent variable provided a model which explained 70% of AER variability (p<0.00001). This same model explained 31% (p=0.01) of AER variability in T2DM patients. Data from T1DM and T2DM patients were pooled together. AER values predicted by glomerular structural parameters were calculated. Squared (observed – predicted) AER values with sign preservation were used for k-means cluster analysis to obtain two maximally different groups A and B. 99% of T1DM and 74% of T2DM patients were clustered in A. All T2DM patients in B had AER>20 μg/min. T2DM patients in A and B with AER>20 μg/min were compared.

Table 1. Renal structural and functional values in microalbuminuric and proteinuric T2DM patients in groups A and B

	T2DM-A	T2DM-B	p-value
AER	91±3	316±3	<0.00001
GFR	92±32	100±27	0.23
Vv(Mes/glom)	0.30±0.09	0.26±0.06	0.03
GBM width (nm)	500±128	458±124	0.13
Sv(PGBM/glom)	0.09±0.02	0.11±0.02	0.0002

Conclusions: T2DM patients are heterogenous in RSFR. While RSFR in 3/4 of T2DM patients resembles that of T1DM patients, 1/4 of T2DM patients have greater AER values despite less severe glomerulopathy (less mesangial expansion and more filtration surface density). Conventional relation of AER values to severity of diabetic glomerulopathy and classification of patients into normo, micro, and macroalbuminuric groups may not be clinically relevant in all T2dm patients.

1379 Acute Transient Arteriopathy in the Early Phase after the Renal Transplantation

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Background: Vascular lesions in early phase of renal allografts are key determinants of outcome. While vascular rejection characterized by infiltration of leukocytes in the arterial walls has a well recognized impact on the graft survival, other vascular lesions have not been well characterized and documented in the literature. We identified a subset of patients with biopsies performed in the peritransplant period which showed proliferative arterial endothelial cell lesions of uncertain clinical significance. We correlated these vascular lesions to renal function and pathology.

Design: The study population consisted of renal allograft biopsies accessioned at the University of Washington, Seattle, from 1/1/ 2000 to 8/31/2008. Based on pathology reports and histology, we identified cases with a unique arterial vasculopathy, characterized by endothelial cell prominence in the absence of vasculitis, arterial sclerosis, or hyalinosis.

Results: Out of 2938 renal allograft biopsies, 11 cases (0.4 %, mean age; 47.2 ± 11.36) showed arterial lesions characterized by endothelial cell swelling, lifting, and vacuolization without diagnostic features of vascular rejection. Mean post-transplantation time was 16.6 ± 14.7 days. All cases were deceased donor kidney transplants. Induction immunosuppressive therapy included anti-thymocyte globulin (91%) and mycophenolate (64%). The indication for biopsy in all the cases was elevated serum creatinine level (5.1 ± 4.3 mg/dl). Three (18 %) biopsies showed coexistent acute cellular rejection and 6 (55%) demonstrated acute tubular injury. C4d staining in peritubular capillaries was negative in all cases. Follow up biopsies were done in 8 cases (mean follow-up time; 94.9 ± 90.4 weeks) at which time the arterial endothelial lesions identified at the initial biopsy had completely resolved in all patients. The follow-up serum creatinine level demonstrated a significant decline (1.45 ± 0.62 mg/dl, $p < 0.005$, mean follow-up time; 107.8 ± 93.9 weeks).

Conclusions: We report an acute arteriopathy occurring in the early phase after the renal transplantation. These lesions are associated with increased serum creatinine level, and are reversible. The pathologic findings and the clinical information indicate that these lesions may represent an acute endothelial injury process that occurs during the peritransplant period, analogous to acute tubular injury.

1380 The Spectrum of Renal Diseases in Biopsies of Patients with Hepatitis C Virus Infection

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Background: Membranoproliferative glomerulonephritis (MPGN) and membranous glomerulonephritis (MGN) are well documented renal complications of Hepatitis C virus (HC) infection. Other immune-mediated GNs have been sporadically linked to HC infection. Patients with HC infection often have risk factors for other renal diseases. The spectrum of diseases in renal biopsies of patients with HC infection has not been systematically studied.

Design: Among 8045 renal biopsies accessioned in our laboratories from 2000-2008, 215 were from patients with HC infection. These biopsies were reviewed. The pathologic diagnoses were correlated with the clinical findings.

Results: Among these 215 biopsies, three diagnostic groups were found. **Group I** (65 biopsies, 30%) included MPGN (54) or MGN (11). **Group II** (11 biopsies, 5%) included several types of immune-mediated GN, which may be etiologically related to HC infection; comprising of diffuse proliferative GN (3), focal segmental proliferative GN (5), and fibrillary GN (3). **Group III** (139 biopsies, 65%) included a large variety of renal diseases which are pathogenetically unrelated to HC infection; they consisted of diabetic nephropathy (51), focal segmental glomerulosclerosis (28), HIV nephropathy (12), non-specific vascular scarring (7), hypertensive nephropathy (6), minimal change disease/ mesangial glomerulonephritis (6), acute tubular necrosis (6), thrombotic microangiopathy (5), lupus nephritis (5), amyloid nephropathy (2), tubulo-interstitial nephritis (2), end-stage kidney disease (2), transplant rejection (2), renal infarct (2), MGN associated with mixed connective tissue disease (1), Ig A nephropathy (1) and chronic ischemic injury (1). There was significant overlapping of the clinical features of patients within each group and among groups.

Conclusions: 1) HC infection is not infrequent in patients submitted to renal biopsies; 2) Although these biopsies often show glomerular lesions directly associated with HC infection such as MPGN or MGN, the majority of them display diseases of prognostic and therapeutic significance, unrelated to HC infection. 3) Clinical features are not helpful in predicting the renal biopsy findings.

1381 Thrombotic Microangiopathy and Humoral Rejection in Renal Allografts

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Background: Thrombotic microangiopathy (TMA) in the transplant kidney may be due to many disorders including humoral rejection (HR), calcineurin inhibitor (CI) toxicity, infections and recurrent disease. Linear peritubular capillary (PTC) C4d deposition is a marker of HR. TMA is described as a feature of HR, however few detailed studies of TMA with PTC C4d are available. Here we hypothesize that HR is a significant risk factor for TMA in the renal allograft.

Design: All consecutive renal allograft biopsies, routinely stained for C4d over a period of thirty-eight months (n=744), were reviewed for TMA and C4d status. All patients received CI (cyclosporine or tacrolimus), mycophenolate mofetil and prednisone immunosuppression.

Results: One hundred thirty biopsies had PTC C4d (focal in 30, diffuse in 100) in the observation period. Of twenty-one biopsies with TMA (2.8%), six had PTC C4d. TMA was observed with greater frequency in C4d+ (4.6%) than in C4d- biopsies (2.4%) (odds ratio 1.9, 95% CI 0.7-5.0, p=0.23). Thrombotic and obliterative arteriolopathy was evident in TMA with PTC C4d (83.3%) and in TMA without PTC C4d (93.3%).

Conclusions: TMA was infrequently observed in renal allograft biopsies. There is no significant increase in risk of TMA in allografts with HR compared to those without HR. Arteriolopathy, a morphologic finding not described in HR, is a prominent feature of TMA with and without PTC C4d. These findings suggest that TMA in HR may be spurious and other possible contributing causes of TMA should be sought in allografts with HR.

1382 IgG1 Subtype Predominance in De Novo Membranous Glomerulonephritis in Renal Allografts, Distinct from Idiopathic Membranous Glomerulonephritis

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Background: *De novo* membranous glomerulonephritis (MGN) after kidney transplantation is known to affect 1 to 2% of renal allografts, usually detected 3 or more years following transplantation. Although proteinuria in these patients may be variable, MGN is certainly an aggravating factor, and can potentially hasten development of chronic allograft nephropathy. IgG subtype staining in native kidney biopsies is becoming a useful aid to define pattern associations with specific etiologies of MGN. IgG4 is the predominant subtype in idiopathic MGN. We report here a distinct staining pattern in *de novo* MGN in renal allografts.

Design: Biopsies with de novo MGN (all detected after one year post-transplant) were retrospectively stained with antibodies to the IgG subtypes. They were semi-quatitatively scored as 0, trace, 1+, 2+ and 3+.

Results: We found ten transplant recipients from January 2004 to August 2008 whose allograft biopsies showed de novo MGN, 7 +/- 4.2 years post-transplant. In two of the patients, cause of end stage renal disease in native kidney was unknown but the biopsies showing MGN were performed four and six years post-transplant respectively. Allograft biopsies from eight patients showed prominent and predominant glomerular capillary staining for IgG1. Only two cases showed concomitant bright staining for IgG4 or IgG3 respectively. Moderate granular staining for IgG1 or IgG2 in tubular basement membrane was seen in two of the cases. All of these 10 patients had nephrotic range proteinuria ranging from 3 to 14 grams/day. Only two patients had concomitant transplant glomerulopathy and two patients had overlapping glomerular collapsing changes along with one or two crescents in their biopsies.

IgG subtype staining in de novo MGN

Patient	Glomerular IgG	IgG1	IgG2	IgG3	IgG4	TBM IgG staining
1	3+	3+	trace	1+	3+	0
2	2+	2+	trace	1 to 2+	trace	2+ IgG1, 1+ IgG2
3	3+	2+	0	trace	1+	0
4	3+	3+	trace	trace	0	0
5	2+	2+	trace	0	0	2+ IgG2
6	1 to 2+	1 to 2+	0	0	0	0
7	1 to 2+	2+	0	0	0	0
8	1+	1 to 2+	0	0	0	0
9	2+	1to 2+	trace	trace	trace	0
10	2+	2+	0	0	0	0

TBM, tubular basement membrane

Conclusions: A consistent IgG1 predominance is noted in de novo MGN, in contrast to IgG4 in idiopathic MGN in native kidney. This may have as yet unknown implications in the pathogenesis. Comparison with biopsies showing recurrent MGN post-transplant is underway.

1383 Erythropoietin (EPO) Protects Against Angiotensin II-Induced Apoptosis and Promotes Angiogenesis of Glomerular Endothelial Cells

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Background: EPO promotes cell survival by binding its receptor, EPO-R, and activating JAK-2 and down-stream signaling cascades in endothelial cells. The aim of the present study was to investigate whether EPO could specifically protect glomerular endothelial cells (GEN) against injury and the possible mechanisms.

Design: Glomerular endothelial cell line was derived from SV40 mice (gift from Dr. Madaio), and divided into four groups: GEN, without any treatment; EPO, treated with 10^{-4} M mouse recombinant EPO; Ang II, treated with 10^{-7} M angiotensin II; Ang II+EPO, treated with both EPO and Ang II. Cells were harvested at 48h.

Results: EPO protected GEN against Ang II-induced apoptosis (Ang II 13.4±0.1% *vs.* Ang II+EPO 6.25±0.15%, P<0.05). This effect of EPO was paralleled by a decrease in caspase 3 activation (Ang II 1.27±0.02 *vs.* Ang II+EPO 0.89±0.16, P<0.05). We also found that Ang II decreased GEN migration (GEN 27.9±4.2% *vs.* Ang II 22.7±1.6, P<0.05), and EPO restored GEN migration (Ang II+EPO 26.9±1.12%, P<0.05). EPO

also preserved VEGF receptor 2 (Flk-1) phosphorylation (Ang II 0.19±0.02 *vs.* Ang II+EPO 0.69±0.14, P<0.05), but there was no effect on endothelial nitric oxide synthase (eNOS) expression (Ang II 0.093±0.01 *vs.* Ang II+EPO 0.059±0.02, pNS). Akt is pivotal in angiogenesis and in promoting cell survival. In our study, Akt phosphorylation was improved by EPO in GEN injured by Ang II (Ang II 0.645±0.01 *vs.* Ang II+EPO 0.884±0.10, P<0.05).

Conclusions: Our data suggest that EPO has direct effects on GEN to promote angiogenesis and protect against Ang II-induced apoptosis, possibly related to Akt phosphorylation and synergistic interactions with VEGF.

1384 Proliferative m-TOR Pathway Is Associated with Injury Tolerance and Repairing in Rat Kidneys

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Background: Renal injury is known to trigger upregulation of many intracellular signal proteins, but role of mammalian target of rapamycin (m-TOR) prolifeative pathway in injured kidneys remains unknown. In this study, protein expression of phosphorylated (p)-mTOR and its down-stream signal p-p70S6K in renal tubules and glomeruli of rat kidneys after renal ischemia-reperfusion injury was evaluated.

Design: Three groups of male adult rats all had right nephrectomy on the day of surgical produre (n = 7 in each group). The left kidney was untouched in group 1. In group 2, the left renal artery was clamped for 20 minutes, followed by 48 hours of reperfusion. A third group of rats received a bolus intraperitoneal injection of nephrotoxic tunicamycin (0.1 mg/kg) one day before the surgery and then underwent the same surgical procedure as in group 2. Serum creatinine was measured in each rat. The rat kidneys were fixed and stained for phosphorylated (p)-mTOR and its down-stream signal p-p70S6K by immunohistochemical method.

Results: Serum creatinine (mg/dl) was significantly higher in group 3 rats (3.82 ± 0.80) than in group 2 rats (0.70 ± 0.07) and group 1 rats (0.37 ± 0.02). Under normal condition, the glomeruli and distal nephron tubules had prominent cytoplasmic expression of p-mTOR and remarkable nuclear expression of p-p70S6K, whereas all segments of proximal tubules had low expression of both markers. In group 2 and group 3 rats, the expression of both markers in glomeruli, S1 segment of proximal tubules and distal nephron tubules was not affected, subjected to the ischemia-reperfusion injury (regardless of tunicamycin treatment). In group 2, both p-mTOR and p-p70S6K were upregualted dominantly in the S3 segment of proximal tubules, following by S2 segment of proximal tubules. These changes were more prominent in group 3, with an additive nephrotoxicity of tunicamycin and corresponding to the morphologic features of acute tubular injury along the vulnerable renal tubules.

Conclusions: Our data suggest that high baseline expression of p-m-TOR and p-p70S6K in glomeruli and distal nephron tubules may be associated injury resistance. The m-TOR proliferative pathway was activated in the injury-vulnerable segment of renal tubules, implying an important role of this proliferative pathway in the repairing process of renal tubules.

1385 Altered Balance of Thymosin β4 and Ac-SDKP Exacerbates Tubulointerstitial Fibrosis

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Background: Our previous study showed that the G-actin sequestering protein thymosin β4 (Tβ4) is remarkably upregulated in the unilateral ureteral obstruction (UUO) model of tubulointerstitial fibrosis. Tβ4 is postulated to be profibrotic but is also degraded by prolyl oligopeptidase (POP) to the anti-fibrotic Ac-SDKP peptide. In this study we investigate whether POP inhibition with or without exogenous Tβ4 affects the early stage of tubulointerstitial fibrosis.

Design: Adult male C57BL/6 wild type mice underwent UUO and were divided into four groups and sacrificed on day 5. Groups were as follows: control UUO without treatment, or with POP inhibitor (S17092, 40mg/kg per day, by gavage), Tβ4 (150μg/d, i.p.), or combination treatment (POP inhibitor plus Tβ4).

Results: Tubulointerstitial injury score was dramatically higher in POP inhibitor and combination treatment groups than untreated UUO (POP inhibitor, 2.87±0.09; combination, 2.94±0.23; control UUO, 2.22±0.19, both p<0.05 vs control). POP activity was significantly lower in POP inhibitor and combination groups (POP inhibitor, 2.06±0.26; combination, 5.73±1.59; control UUO, 26.62±2.74 pmol/min*mg tissue, both p<0.05 vs control). Compared to untreated UUO control group, neither injury score nor POP activity was different in Tβ4 group (2.19±0.13 and 21.19±1.61 pmol/min*mg tissue, respectively). There was no difference in Tβ4 expression among all four groups by western blot analysis (POP inhibitor, 1.15±0.17; combination, 0.84±0.09; control UUO, 1.02±0.05; Tβ4, 0.83±0.06). However, the balance of Tβ4 vs Ac-SDKP level in both serum and tissue could be shifted.

Conclusions: Our study suggests that POP inhibitor with or without Tβ4 may cause tubulointerstitial fibrosis by inhibiting degradation of Tβ4 and thus also Ac-SDKP formation. We propose that the balance of Tβ4 and Ac-SDKP may play a pivotal role in determining renal interstitial fibrosis.

Liver & Pancreas

1386 Precancerous Bile Duct Pathology in Sporadic Hilar Cholangiocarcinoma

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Background: Most cholangiocarcinomas (CCAs) arise in the hilum, an anatomic region that often renders them surgically unresectable. This has hindered the study of presumed precursor lesions (metaplasia and dysplasia) in noncancerous bile ducts of the hilum and intrahepatic parenchyma.

Design: We studied 29 patients with sporadic hilar CCA who underwent neoadjuvant chemoradiation followed by liver transplantation from 1994-2007. After reevaluating the preserved gross liver explants, we took multiple (11) casettes of large hilar and intrahepatic bile ducts. Ducts were scored for the following histologic features: metaplasia (intestinal, pyloric, and mucinous/foveolar), dysplasia (low or high grade, number of dysplastic ducts, and hilar or intrahepatic location), and cancerization (malignant biliary epithelium near invasive CCA). These results were compared with two previously published control groups: 1) 28 patients with PSC-associated hilar CCA who received chemoradiation and liver transplant, and 2) 65 noncirrhotic liver transplants without CCA in patients >21 yr.

Results: Twenty-eight (97%) patients were Caucasian and 1 (3%) Asian; none had known predisposing conditions for CCA (choledochal cysts, hepatolithiasis, etc). Residual CCA was detected in 22 (76%) and ductal cancerization in 3 (10%). They had low rates of intestinal metaplasia (10%) but high rates of pyloric metaplasia (90%), mucinous metaplasia (93%) and bile duct dysplasia (55%). Of 16 cases with dysplasia, 10 involved both hilar and intrahepatic ducts and 6 involved intrahepatic ducts only. Comparison with PSC-associated CCA and non-CCA liver explants is shown below.

Bile Duct Pathology in Hilar CCA

	Age	Gender	Biliary Metaplasia			Biliary Dysplasia	
			Intestinal	Pyloric	Mucinous	Any	High-grade
Sporadic hilar CCA (n=29)	52 yr (22-64)	62% M	10%	90%	93%	55%	17%
PSC with hilar CCA (n=28)	47 yr (p=0.09)	71% M (p=1)	39% (p=0.015)	75% (p=0.2)	89% (p=0.7)	82% (p=0.045)	57% (p=0.003)
Noncirrhotic without CCA (n=65)	47 yr (p=0.08)	63% M (p=1)	8% (p=0.7)	9% (p<0.001)	72% (p=0.03)	15% (p<0.001)	0% (p=0.002)

*P values are in comparison to the sporadic CCA group

Conclusions: The presence of intestinal metaplasia and the presence and extent of bile duct dysplasia are significantly lower in sporadic vs. PSC-associated hilar CCA, suggesting a "field effect" in PSC. Pyloric metaplasia and dysplasia of noncancerous bile ducts best distinguish CCA from non-CCA cases.

1387 Serum and Tissue FGL2 as a Surrogate for Treg Cells Activity in Patients with Chronic Viral Hepatitis C (HCV)

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Background: CD4+/CD25+/FoxP3+ T cells (Tregs) a subset of T cells have been implicated in the suppression of specific T cell-mediated immune responses to HCV. Tregs activity appears to correlate with more aggressive disease in HCV infected patients, suggesting that identification of these cells in serum and tissue could be an important prognostic marker. Hitherto FoxP3 was thought to be the most specific marker for Tregs, but recent data has cast doubts on their specificity. Data genearted in our lab our from mice studies supports the hypothesis that Fibrinogen-like protein (FGL2)/fibroleukin is an important effector molecule of Tregs. We have therefore tested the hypothesis that measurement of FGL2 as surrogate marker for Tregs activity in serum and liver tissue has prognostic value in predicting disease severity and response to antiviral therapy.

Design: We measured serum levels of human FGL2 using a highly sensitive and reproducible ELISA method in healthy individuals (controls) and post-transplant HCV-patients. The latter were grouped according to HCV genotype and response to antiviral therapy, to determine whether levels of FGL2 can be a useful prognostic marker of response to IFN therapy. FGL2 and FGL2/FoxP3 double staining was performed on randomly selected liver explants of these HCV patients.

Results: Serum levels of FGL2 were significantly higher in HCV patients (13.1±4.7ng/ml) than control (5.8±1.3 ng/ml p=0.007). HCV genotype 1 infected patients had higher levels of FGL2 (13.11+2.4ng/ml) than genotype 2/3 patients (6.1+1.4ng/ml) (p=0.04). Serum FGL2 levels were lower in patients who responded to anti-viral therapy than non-responders (3.5+1 ng/ml p =0.001) and was similar to non infected controls. FgL2 immunostaining demonstrated more positive cells among portal and lobular infiltrates in HCV patients with genotype 1 >> other genotypes. FoxP3/FGL2 co-staining showed that the majority of FoxP3+cells were not FGL2 positive, supporting the views that not all FoxP3+ cells are Tregs.

Conclusions: In HCV patients high Serum FGL2 levels, as surrogate for Tregs activity, mirror histopathologic findings and both correlate with known risk factors for progression (HCV genotype 1 and poor response to IFN). Serum and/or histologic demonstration of FGL2 could provide useful prognostic information in the management of HCV-infected patients. We have an ongoing prospective study to investigate the predictive value of serum and tissue FGL2 in HCV infection.

1388 Histologic Features of Non-Alcoholic Fatty Liver Disease in Young Adults

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Background: The clinical and pathological findings of non-alcoholic fatty liver disease (NAFLD) have been well-characterized in adults, and more recently a unique histologic pattern of NAFLD ("Type 2") has been described in pediatric patients. Most of the adult