Although they are present in all eukaryotes, GPIs are essential in fungi for their role in crosslinking of the fungal cell wall. Previous work has shown that the GPI biosynthetic pathways are different in fungi and humans. Moreover, the fungal and human GPI biosynthetic pathways are regulated by different mechanisms. Previous research in our laboratory has shown that the Ras protein inhibits the initial enzyme in yeast GPI biosynthesis. We set out to identify new yeast mutants defective in GPI assembly using a colony sectoring screen. We then tested the ability of these mutants to carry out the first step of GPI assembly: the transfer of *N*-acetylglucosamine from the donor molecule UDP-GlcNAc to the acceptor lipid phosphatidylinositol to form the reaction product GlcNAc-PI.

**Results:** We identified the *Saccharomyces cerevisiae* mutant *swi1* in a colony sectoring screen for mutations synthetically lethal with *gpi1*, a mutation affecting the first step of GPI biosynthesis. We show that yeast *swi1* mutants are unable to efficiently execute the first and committed step of the GPI biosynthetic pathway, demonstrating that the Swi1 protein is required for effective synthesis of GPI anchors. We also demonstrate that *swi1* cells, which are defective in their ability to synthesize inositol, are unable to form GlcNAc-PI even when supplemented with excess inositol.

**Conclusions:** The identification of the Swi1 protein as an enhancer of GPI synthesis in yeast, but not humans, suggests divergent regulatory mechanisms that may be targeted for the development of new antifungal agents.

# Kidney

### 1322 Multinucleated Giant Cells in Anti-Glomerular Basement Membrane Disease (AGBM)

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**Background:** Inflammatory cells are numerous in crescentic forms of glomerular disease. Multinucleated giant cells (MGC), however, are uncommon but have been described in several case reports of AGBM disease and ANCA/systemic vasculitis-associated crescentic glomerulonephritis (GN).

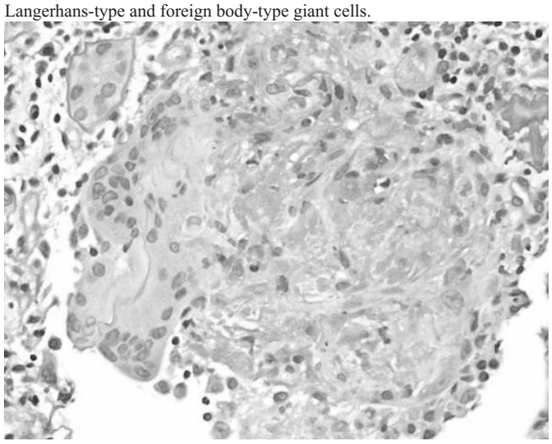
**Design:** Twenty-six cases of AGBM disease and 50 cases of non-AGBM crescentic GN (32 pauci-immune; 12 systemic lupus erythematosus, 6 immune complex-associated) were reviewed and the percentage of glomeruli containing MGC were determined.

**Results:** 18/26 cases of AGBM contained MGC (69%), compared with 2/50 non-AGBM diseases (4%). In the 2 non-AGBM cases with MGC, 1 MGC was noted in a single glomerulus in each case. In AGBM disease the percentage of glomeruli containing MGC ranged from 10-100% (see table).

Multinucleated Giant Cells in Crescentic GN

| | | | % of Crescentic Glomeruli with MGC | | |
|---|---|---|---|---|---|
| Disease | # Cases | # with MGC | >10% | >20% | >40% |
| AGBM | 26 | 18 | 18 cases | 15 cases | 5 cases |
| Non-AGBM | 50 | 2 | 0 | 0 | 0 |

Affected glomeruli ranged from acute necrotizing lesions with cellular crescents to fibrocellular and fibrous crescents. MGC were identified in completely sclerotic glomeruli in 6 cases. One case had 1 interstitial focus of MGC not clearly related to a glomerulus. The number of MGC per glomerulus ranged from 1 to 4 and included Langerhans-type and foreign body-type giant cells.



No basement membrane or matrix remnants were detected within the MGC on PAS or silver stains. Immunoperoxidase stain for CD68 was positive and cytokeratin was negative in the MGC in the cases tested.

**Conclusions:** (1) MGC are common in AGBM and rare in non-AGBM crescentic diseases (2) The presence of MGC strongly supports a diagnosis of AGBM in the absence of serologic and immunofluorescence data (3) Formation of MGC in AGBM likely reflects the immunopathogenesis of AGBM disease rather than the presence of a necrotizing event since MGC are usually absent in other crescentic diseases.

### 1323 Prognostic Pathological Features in Renal Biopsies in Scleroderma Renal Crisis

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**Background:** Scleroderma renal crisis (SRC) is an idiopathic microangiopathic disorder that affects the kidneys in a subset of patients (pts) with systemic sclerosis. Aggressive therapy with ACE inhibitors may prevent permanent renal failure in some pts. This study was designed to identify pathological prognostic factors and to investigate the role of a C4d in SRC.

**Design:** We retrospectively reviewed the pathology and the clinical records of 17 pts that underwent kidney biopsies during SRC at the University of Pittsburgh Medical Center between 1990 and 2007. Multiple histological features were assessed semi-quantitatively (0-3+) or as percentages. C4d staining of peritubular capillaries (PTC) and arteries was assessed in SRC pts (n=10) and controls (n=8) using a semi-quantitative scoring system (0-3+). Pts recovering renal function (group A: n=7) and those remaining in renal failure or dying (group B: n=10) were compared.

**Results:** No difference was found comparing age, sex, or presenting serum creatinine, however, a trend toward a difference in diastolic blood pressure (116 ± 13 mmHg group A vs 99 ± 21 mmHg group B) was noted. The percentage of thrombosed arteries/infarction was significantly higher in group B (28.5 ± 19.2) than group A (5.6 ± 12.3; p=0.017), as well as glomerular ischemic collapse (2.9 ± 0.3 group B vs 1.43 ± 0.79 group A; p=0.001). Also, group B pts showed a trend toward more severe acute tubular injury (1.8 ± 0.63 vs 1.14 ± 0.69; p=0.1) and fibrinoid necrosis (0.7 ± 0.95 vs 0 ± 0; p=0.195). Other histologic parameters assessed were similar between the two groups. C4d score was higher within arteries in scleroderma pts (2.11 ± 1.27) compared with controls (0.5 ± 0.9; p=0.023), and trended to be higher within PTCs [1.2 ± 1 scleroderma vs 0.25 ± 0.7 controls; p=0.055]. Moreover, C4d score trended to be higher in group B within PTC (1.67 ± 0.81 group B vs 0.5 ± 1 group A, p=0.11) as well as arteries (2.8 ± 0.5 group B vs 1.25 ± 1.5 group A; p=0.11).

**Conclusions:** The presence of arteriolar thrombosis/infarction and severe glomerular collapse in SRC renal biopsies correlates with increased risk of failure to recover renal function and death. C4d staining was more frequently detected in both PTC and arteries in SRC pts compared to controls, suggesting an ongoing antibody-mediated injury, although no immune complex deposits were identified. C4d staining tended to be associated with a worse prognosis, however, a larger study is needed to more conclusively address this issue.

### 1324 Thymosin β4 (Tβ4), a Marker and Potential Mediator of Progressive Sclerosis, Is Increased in Chronic Allograft Nephropathy (CAN)

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**Background:** Tβ4 is a G-actin-sequestering protein with myriad functions, including cell differentiation, angiogenesis, wound healing, and regulation of inflammation. We have identified upregulated Tβ4 by proteomic analysis in a rat model of focal segmental glomerulosclerosis, and as a necessary intermediary for angiotensin II-induced upregulation of plasminogen activator inhibitor (PAI-1). PAI-1 inhibits both fibrinolysis and proteolysis and is linked to progressive scarring. We have shown previously that PAI-1 is increased in CAN (Revelo et al, NDT 2005). We therefore aimed to investigate whether Tβ4 is also increased in these cases of CAN, and is linked to sites of PAI-1 expression.

**Design:** 97 renal transplant biopsies or nephrectomy specimens from 75 patients with CAN were studied and compared with transplant (Tx) biopsies without CAN (n=8) and normal native kidney (n=7). Tβ4 expression and macrophage localization were detected by immunohistochemistry. Glomerular cell, tubular, interstitial, and vascular staining was scored as cells/glomerulus or on a 0 - 3 scale.

**Results:** Native and normal Tx kidneys showed Tβ4 staining only in collecting duct epithelial cells, rare peritubular capillaries and rare glomerular macrophages. In CAN, podocytes, non-podocyte glomerular cells, and parietal epithelial cells showed increased Tβ4 vs normal and normal Tx. Tubular Tβ4 staining was 0.92±0.48 and vascular staining was 12.9±16.9% in CAN and minimal in normal and native and normal Tx. Interstitial Tβ4 was dramatically increased in CAN, 2.69±0.47, correlating with fibrosis, and was absent in normal native and normal Tx. Interstitial and glomerular Tβ4 were both contributed to by infiltrating macrophages. Increased Tβ4 was present in particular in biopsies with increased PAI-1.

**Conclusions:** Tβ4 immunostaining is significantly increased in glomeruli, interstitium and vessels in CAN compared to native and transplant control kidneys, and is linked to fibrosis and PAI-1 expression. We speculate that Tβ4 contributes to fibrosis at least in part by augmenting PAI-1.

### 1325 In-Situ B Cell Immune Responses Contribute to the Pathogenesis of Human Lupus Nephritis

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**Background:** We have previously demonstrated significant quantities of interstitial B lymphocytes and plasma cells (PC) in human lupus nephritis (LN) kidneys. We hypothesize that analyzing the immunoglobulin (Ig) repertoire from interstitial PCs of LN kidneys may give us additional insight into this autoimmune disease.

**Design:** 70 kidney biopsies from LN patients were analyzed. The interstitial inflammation was divided into the following patterns: 1) diffuse and scattered; 2) T:B cell aggregates, or 3) germinal center (GC) formation, which was correlated with the presence of tubular basement membrane (TBM) immune complex deposition. The frozen tissue from a GC and another biopsy with T:B cell aggregates were identified. The sections were immunohistochemically stained with anti-CD38 and individual and small groups of PCs were isolated by laser capture microdissection (LCM) using the Arcturus Pixcell II system. RT-PCR was performed directly on the LCM caps and IgG primers to the heavy and light chains were used. The products were sequenced and the antibody sequences were analyzed. The germline sequences were identified by aligning sequences in IMGT/V-QUEST.

**Results:** Six of 33 (18%) biopsies with the diffuse and scattered inflammatory pattern compared with 21 of 37 (57%) biopsies with either T:B cell aggregates or GCs contained TBM immune complex deposition (p=0.0006). Sequence analysis of Ig expression of

PCs from a GC and adjacent areas demonstrated a highly restricted repertoire that likely arose from local clonal expansion. Furthermore, the pattern of somatic hypermutation in GC expressed Igs indicated that they had undergone antigen-driven selection. In contrast, clonal expansion but not antigen-driven somatic hypermutation was observed in PCs isolated from and around a T:B cell aggregate from a different patient.
**Conclusions:** The presence of either T:B cell aggregates or GCs was strongly associated with more severe tubulointerstitial inflammation and the deposition of TBM immune complexes. In addition, the presence of local antigen-driven somatic hypermutation suggests that local autoantibody production contributes to the pathogenesis of human LN and these autoantibodies may be targeting intrinsic renal antigens.

### 1326 Expression of B Cell Associated Transcripts and Immunoglobulin Transripts Occurs Only in Late Renal Allograft Biopsies and Is Related to the Inflammatory Burden

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**Background:** The significance of B cell infiltrates for outcome in renal allograft rejection remains controversial.
**Design:** Using microarrays, we identified B cell associated transcripts (BATs) with high expression in B cells. Because of their high expression in plasma cells, we excluded immunoglobulin transcripts from this set and summarized them separately (BATs-Ig). We compared expression of BATs and BAT-Igs to histologic lesions, diagnosis, and renal function in 175 renal allograft biopsies taken for clinical indication.
**Results:** To confirm that the transcript sets reflects the presence of B cells in the graft, we stained selected biopsies (n = 32 with rejection) for CD20. BAT scores but not BAT-Igs correlated significantly with the number of $CD20^+$ B cells (r = 0.42); both BAT and BAT-Ig scores correlated with the number of lymphoid clusters (r = 0.56, r = 0.43). Expression of BATs and BAT-Igs was increased in biopsies with rejection compared to non-rejecting biopsies. However, there was no significant difference between T cell mediated and antibody mediated rejection. BAT and BAT-Ig expression was not unique to rejection but was also observed in some biopsies without rejection (calcineurin inhibitor toxicity, recurrent GN). Biopsies with BK virus nephropathy had variable degrees of BAT and BAT-Ig expression. BATs and BATs-Ig correlated strongly with time post transplant (r = 0.34, r = 0.61, respectively). Interestingly, biopsies taken within 5 months post transplant did not express BAT-Igs, indicating that plasma cells do not home to the graft during this early period. In a univariate analysis, BAT and BAT-Ig expression correlated with the degree of interstitial inflammation, interstitial fibrosis, and tubular atrophy. In a multiple regression, only time and degree of interstitial inflammation remained significant parameters related to BAT and BAT-Ig expression. There was no significant relationship between expression of BATs or BAT-Igs and kidney function independent of time post transplant.
**Conclusions:** In summary, BAT and BAT-Ig expression was time dependent and correlated with the degree of interstitial inflammation but was not unique to rejection, indicating that B cells may home to the graft non-specifically in response to injury. Our study does not support a significant role for B cell infiltrates for allograft function. B cells and plasma cells accumulating in renal allografts are probably a non specific consequence of renal inflammation and injury.

### 1327 IgA Nephropathy in the Triethnic Population of New Mexico

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**Background:** IgA nephropathy (IgAN) is the most frequent glomerulonephritis around the globe,and has a preponderance for certain racial/ethnic groups. Because we noted many IgAN cases with adverse histologic features on our renal biopsy service, we wondered if this may relate to one of the minority populations in our state. Our aim was to review clinico-pathologic data in a cohort of IgAN cases biopsied throughout the state of New Mexico, and to determine if there are any differences among the three major racial/ethnic populations.
**Design:** Renal biopsies from 112 consecutive cases of IgAN were identified retrospectively. Cases from all over the state of New Mexico were received at our institution. Clinical data was retrieved and biopsies were reviewed for histo-pathologic variables including glomerular pathologic patterns and interstitial fibrosis.
**Results:** When compared to their demographic representation in New Mexico, American Indians were twice as frequent in our IgAN cohort, with the reverse finding for Non-Hispanic Whites. Hispanics more frequently developed the nephrotic syndrome than American Indians or Non-Hispanic Whites. Endocapillary proliferative glomerulonephritis was the most common glomerular abnormality, followed by the FSGS-like pattern, and the mesangio-proliferative pattern typically expected in IgAN. An FSGS-like pattern was more frequent in the American Indian and Hispanic groups than in Non-Hispanic Whites. Moderate to severe interstitial fibrosis was most common in the American Indian group.
**Conclusions:** The American Indian and Hispanic populations were at increased risk for IgAN when compared to the general population of New Mexico. Clinico-pathologic features of more severe disease and more advanced chronic changes were seen in the American Indian and Hispanic populations. These findings indicate that American Indians and Hispanics are likely to have a more aggressive disease course and should be biopsied early when IgAN is suspected.

### 1328 Accumulation of Interstitial B Lymphocytes and Plasma Cells in Human Pauci-Immune Crescentic Glomerulonephritis

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**Background:** Anti-neutrophil cytoplasmic antibodies (ANCA) have been recently demonstrated to be pathogenic in both an animal model and human pauci-immune crescentic glomerulonephritis (GN). Immunophenotypic characterization of the interstitial inflammatory cells in renal biopsies of these patients has not previously focused on B lymphocytes or plasma cells. We hypothesized that activated B cells and plasma cells in kidneys with crescentic GN may play a significant role in the local production of pathogenic autoantibodies.
**Design:** 33 consecutive human renal biopsies of pauci-immune crescentic GN were studied. Standard immunohistochemistry was performed on paraffin tissue sections using monoclonal antibodies to CD3, CD20, CD45, and CD138. The percentage of interstitial $CD45^+$ cells staining for CD3, CD20, and CD138 was assessed. The extent of interstitial inflammation, tubular atrophy, and interstitial fibrosis was graded according to area involved as mild (1-25%), moderate (26-50%), or severe (greater than 50%). The percentage of crescent formation and global glomerulosclerosis was also assessed.
**Results:** There were varying degrees of interstitial inflammation. As expected, $CD3^+$ T cells predominated, representing 43.1% (range: 10.7-79.6%) of the total $CD45^+$ cells. $CD20^+$ B cells and $CD138^+$ plasma cells were present in all biopsies and comprised 6.3% (range: 1.2-21.4%) and 8.5% (range: 0.3-57.5%), respectively, of all $CD45^+$ cells. In 8 (24%) of the biopsies, >20% of the $CD45^+$ cells were of the B cell lineage ($CD20^+$ and $CD138^+$). Prominent aggregates of plasma cells were often present around Bowman's capsules and atrophic tubules. There was no correlation between the percentages of interstitial B cells or plasma cells to either clinical parameters (ANCA titer) or histopathologic parameters (the extent of crescentic glomerular injury or interstitial fibrosis).
**Conclusions:** B cells and plasma cells are present in significant quantities within the kidneys of ANCA-associated, pauci-immune crescentic GN, in a location to contribute to the pathogenesis of this autoantibody-mediated disease. However, the absence of correlation between the percentages of B and plasma cells with any histopathologic parameters suggests that the inflammatory cells may be present in a non-specific manner.

### 1329 Interstitial Regulatory T Cells in Human Pauci-Immune Crescentic Glomerulonephritis

*KJ Henriksen, SM Meehan, A Chang.* University of Chicago Medical Center, Chicago, IL.
**Background:** The pathogenesis of pauci-immune crescentic glomerulonephritis, a chronic inflammatory autoimmune disease, is poorly understood. A majority of cases are characterized by the presence of anti-neutrophil cytoplasmic antibodies (ANCA). An imbalance between regulatory T cells and effector T cells may predispose individuals to this autoimmune disease. We hypothesized that a relationship might exist between the percentage of interstitial regulatory T cells and disease activity in the kidneys of patients with pauci-immune crescentic glomerulonephritis.
**Design:** 33 consecutive human renal biopsies of pauci-immune crescentic glomerulonephritis were studied. Standard immunohistochemistry was performed on paraffin tissue sections using monoclonal antibodies to CD3, CD45, and the transcription factor forkhead box P3 (FOXP3). The percentage of interstitial $CD45^+$ cells staining for CD3 (membranous stain) and FOXP3 (nuclear stain) was assessed. The extent of interstitial inflammation, tubular atrophy, and interstitial fibrosis was graded according to area involved as mild (≤25%), moderate (26-50%), or severe (>50%). The percentage of crescent formation and global glomerulosclerosis was also assessed. These histopathologic parameters were correlated with the percentage of interstitial regulatory T cells.
**Results:** $CD3^+$ T cells were the predominant lymphocyte subset in the renal biopsies and represented on average 43.1% (range: 10.7-79.6%) of the total interstitial $CD45^+$ cells. The $FOXP3^+$ regulatory T cells were found diffusely throughout the renal interstitium, comprising on average 3.0% (range: 0.1% to 17.8%) of the CD45+ interstitial lymphocytes and 6.4% of the CD3+ T cells (range: 0.2-31.2%). There was no correlation between the percentage of interstitial regulatory T cells to either clinical parameters (ANCA titer) or histopathologic parameters (crescentic glomerular injury, interstitial inflammation or fibrosis, or vasculitis). Of interest, an average of 7.6% and 5.3% regulatory T cells was found in biopsies with <50% (n=16) versus ≥50% (n=17) crescentic glomerular injury, respectively, but this inverse relationship was not statistically significant (p=0.24).
**Conclusions:** Significant quantities of interstitial FOXP3+ regulatory T cells were observed in our study, but their percentages did not demonstrate significant correlation with any histopathologic parameters of disease activity or chronicity in renal biopsies of pauci-immune crescentic glomerulonephritis patients.

### 1330 Membranoproliferative Glomerulonephritis (MPGN) Associated with Chronic Lymphoproliferative Disorders

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**Background:** Membranoproliferative glomerulonephritis (MPGN) is a histopathologic term that describes glomerular mesangial and segmental to global endocapillary proliferation with mesangial and subendothelial immune deposits and subendothelial basment membrane duplication. Membranoproliferative glomerulonephritis is thought to arise as a consequence of immune dysregulation caused by prolonged immune stimulation (hepatitis C, for example), and the presence of cryoglobulins in circulation. Chronic lymphoproliferative disorders (CLPD) can be assoicated with MPGN, with or without cryoglobulinemia.
**Design:** Over a ten year period (1997-2007), 304 cases of MPGN in adults (> 30 years of age) were diagnosed. The cases were reviewed for an association with CLPD. The review included demographic and clinical data, laboratory data, biopsy material and special stains, including immunohistochemical stains.
**Results:** Of the 304 adult patients with MPGN, 13 (4%) were found to have evidence of CLPD, either on the diagnostic kidney biopsy or biopsy of other sites (ie. bone marrow or lymph node). The 13 patients with CLPD and MPGN included 4 females and 19 males, with an age range of 45 to 85 years (median 72 years). Three patients had documented chronic hepatitis C infection at the time of biopsy and five patients

had cryoglobulinemia. The most common type of CLPD associated with MPGN was small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL), present in seven patients. One patient had a small B cell lymphoma with the differential diagnosis of SLL/CLL vs. mantle cell lymphoma. Two patients had low grade B cell lymphoma with plasmacytic differentiation. The remaining three patients had marginal zone, mantle cell lymphoma, and low grade B cell lymphoma NOS, respectively. Renal biopsy slides from ten patients were available for re-review, and in all cases, the diagnosis of MPGN was confirmed. Biopsies from nine of these ten patients also showed renal involvement by the CLPD with focal to diffuse interstitial lymphocytic infiltrates. Four patients had morphologic evidence of cryoglobulin deposition within glomeruli.

**Conclusions:** Our study shows that MPGN is infrequently associated with CLPD. However, the recognition of renal involvement by a malignant lymphoma, concomittant with MPGN is important both therapeutically and prognostically. The mechanism of developing MPGN in the context of CLPD likely involves immune dysregulation with immune complex or free immunoglobulin deposition in the background of genetic susceptibility.

### 1331 Gene Expression Profiling of Diabetic Nephropathy Using Paraffin Embedded Nephrectomy Samples

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**Background:** Gene expression analysis in medical diseases of the kidney is often limited by the paucity of available frozen renal biopsy tissues. Recent studies have successfully used gene microarray technology in formalin fixed paraffin embedded (FFPE) tumors. Diabetic nephropathy (DN) is common and can be seen in the non-neoplastic parenchyma in nephrectomies performed for renal tumors and thus can be ideally studied by gene expression analysis.

**Design:** The pathology database at SUMC was searched (Jul 1995-Jul 2007) for a diagnosis of DN in nephrectomies performed for carcinoma. The medical records were reviewed and the diagnosis of type II diabetes mellitus was confirmed. Nine cases with adequate normal parenchyma for analysis were selected, along with patient age and year of surgery (i.e. age of the block)-matched normal kidneys (n=3). In addition, a normal frozen kidney sample from a nephrectomy was included in the analysis. The serum creatinine in DN group ranged from 0.7 to 1.8 mg/dl (mean 1.2). RNA extracted from the FFPE kidneys was subjected to one round of amplification and used for microarray hybridization. The microarrays used in the study are the HEEBO arrays (SFGF, Stanford). For the analysis, spots with a red/green fluorescence ratio of 1.5 over background and genes with over four fold mean expression levels in at least 3 arrays were included.

**Results:** Microarray analysis of DN kidneys identified 842 differentially expressed genes compared to normal kidneys. Unsupervised hierarchical cluster analysis clustered all the samples into two major groups. Most DN (n=7) cases clustered together, while two mild DN cases clustered with normal kidneys. Interestingly, the normal frozen kidney clustered with normal FFPE kidney. For significance analysis of microarrays, the 7 DN kidneys that tightly clustered in a group were compared to 4 normal kidneys. This identified 151 upregulated and 254 downregulated genes in DN samples. When compared to normal, DN kidneys showed increase in expression of insulin-like growth factor binding proteins, cell cycle and chromatin remodeling genes and decreased expression of apolipoproteins, mitochondrial cytochrome genes and subset of cyclins.

**Conclusions:** In summary, most DN kidneys are characterized by gene expression signature that distinguishes them from normal kidneys. This study confirms that FFPE tissues can be adequately utilized for gene expression profiling of medical renal diseases.

### 1332 Renal Mucosa-Associated Lymphoid Tissue Lymphoma (MALT) in Native Kidney Biopsies

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**Background:** Diagnosis of lymphoma on native kidney biopsies submitted for evaluation of medical renal disease is uncommon with most cases representing secondary involvement by systemic disease. Primary renal lymphomas limited to the kidney are rare and can pose a diagnostic challenge. We present clinical, laboratory and renal biopsy findings in 3 cases of renal MALT lymphoma, two of them diagnosed during workup for renal failure.

**Design:** Three cases of MALT lymphoma were diagnosed on renal biopsy at Tufts-NEMC in the past two years. Two were primary renal MALT and one was associated with oral MALT. Renal biopsies were processed according to routine protocols including light microscopy (LM), immunofluorescence (IF) and electron microscopy (EM). Immunohistochemical studies and cytogenetic analysis by fluorescent in situ hybridization (FISH) were performed on formalin fixed paraffin embedded material with commercially available antibodies and probes. Clinical information was obtained from medical records.

**Results:** The patients with primary MALT lymphoma were two men 55 and 78 years old presenting with renal failure. One had a history of high-grade papillary transitional cell carcinoma of the bladder and the other history of cutaneous leukocytoclastic vasculitis. LM examination in both patients revealed diffuse global membranoproliferative glomerulonephritis with subendothelial and mesangial deposits including tubular structures consistent with cryoglobulins (EM) along with granular IgM and C3 in the capillary loops and arterial walls (IF). The interstitium showed small lymphocytic infiltrate of CD20 positive B-cells expressing IgM and negative for CD5, CD10 and Cyclin D1. Subsequent workup showed clonal serum IgM and bone marrow involvement by lymphoma. The third patient was a 55 years old woman with recently diagnosed oral MALT lymphoma. Her biopsy was composed exclusively of lymphoma with identical immunophenotype. FISH analysis for MALT1 gene rearrangements was negative in all three cases.

**Conclusions:** Primary renal MALT lymphoma could be more frequent than reported. Workup of cryoglobulinemic glomerulonephritis should include immunophenotyping of the lymphoid infiltrate on the biopsy, serum protein electrophoresis and urine immunofixation. Renal MALT lymphoma is immunophenotypically identical with other extranodal MALT lymphomas and does not show rearrangements of the MALT1 gene on chromosome 18 by FISH.

### 1333 Lymphangiogenesis in Renal Diseases

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**Background:** Lymphatics are important for fluid homeostasis and maintenance of chronic inflammatory reactions. Lymphangiogenesis has been recently described in chronic renal allograft failure. The role of lymphatics in renal diseases has not been investigated.

**Design:** Renal biopsies performed for various medical renal diseases were reviewed. Immunohistochemical stain for D2-40, a specific marker for lymphatic endothelial cells, was performed on 48 cases with adequate tissue for examination. Normal kidney tissue from nephrectomies performed for renal neoplasms was used as control. Lymphatic density in each case was calculated as the mean number of lymphatics per high power field.(HPF) The location and the number of lymphatics were compared with the normal controls, and correlated with the severity of interstitial fibrosis and inflammation.

**Results:** Renal cortical tissue was present in all the cases and with medullary tissue in 12 cases. The diagnoses and average lymphatic densities per HPF are shown.

| Diagnosis | Number of Cases | Lymphatic density/HPF |
|---|---|---|
| Focal segmental glomerulosclerosis | 14 | 22.4 |
| Diabetic nephropathy | 6 | 17.1 |
| Immune complex glomerulonephritis | 7 | 14.1 |
| Arterionephrosclerosis | 5 | 6.4 |
| Minimal change nephropathy | 4 | 4.95 |
| HIV Nephropathy | 2 | 13 |
| Crescentic pauci-immuneglomerulonephritis | 3 | 9.5 |
| Chronic allograft nephropathy | 2 | 16.3 |
| Thrombotic microangiopathy | 2 | 6.8 |
| Light chain deposition disease | 1 | 12 |
| Renal amyloidosis | 1 | 24 |
| Hereditary nephritis, Alport's type | 1 | 0 |
| Normal kidney | 10 | 2 |

The normal kidneys showed lymphatics around the arcuate and interlobular arteries. No lymphatics were seen in the medulla. Lymphangiogenesis was seen in the interstitium in almost all the cases. Six of twelve cases showed lymphatics in the medulla. One case of crescentic glomerulonephritis also had lymphatics around the glomeruli. Lymphangiogenesis correlated with the extent of interstitial fibrosis and interstitial inflammation, but independent from diagnostic categories. Lymphangiogenesis was also seen in the absence of interstitial fibrosis in minimal change nephropathy.

**Conclusions:** Lymphangiogenesis, often pronounced, is seen in many renal diseases. Its severity correlates with interstitial fibrosis and inflammation, regardless of the disease processes, indicating a fundamental role in renal injury, such as clearing of the interstitial fluid, or channeling of kidney-specific antigens to lymphoid organ for the initiation of immune-mediated renal injury.

### 1334 Anti VEGF Therapy: A New Cause of Unusual Glomerulopathies?

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**Background:** Therapies to block VEGF have gained considerable clinical interest for treating RCC and other malignant neoplasms. Anti-VEGF drugs include Bevacizumab, a recombinant human monoclonal antibody directed against VEGF, and Sunitinib, a tyrosine kinase inhibitor that blocks VEGF and PDGF receptor signaling. Side effects, seen in 5-50% of patients, include hypertension and proteinuria. Although VEGF signaling is important for maintaining the glomerular filtration barrier, so far only 2 anecdotal case reports have described glomerular changes in renal biopsies from treated patients.

**Design:** Retrospective morphologic and clinical analysis of 3 patients under anti-VEGF therapy who underwent renal biopsy for nephrotic proteinuria.

**Results:** Clinical: Patient #1 is a 71 y.o. woman with metastatic RCC who developed pleural effusions and nephrotic proteinuria after completing 10 cycles of Sunitinib therapy. Patient #2 is a 70 y.o. woman with metastatic RCC who developed nephrotic proteinuria after 4 months of Bevacizumab therapy. Patient #3 is a 80 y.o. male with metastatic RCC who developed acute nephrotic proteinuria with renal failure after 18 months of Sunitinib therapy. LM: All 3 biopsies showed similar findings with diffuse glomerular capillary wall thickening and segmental duplication, capillary hyalinosis, mesangiolysis, and segmental sclerosis. Fibrin thrombi or typical signs of thrombotic microangiopathy were not noted. IF: See table 1 (cw: capillary wall, m: mesangium). EM: Glomeruli showed GBM remodeling with rudimentary duplication, segmental marked endothelial cell injury, and segmental foot process effacement. Partially hyaline-like dense material was seen in subendothelial spaces in all biopsies. Rare mesangial and GBM dense deposits were seen in patient #1.

Biopsy findings by IF

| IgG | IgA | IgM | C3 | Fibrin | C1q | Kappa | Lambda |
|---|---|---|---|---|---|---|---|
| **1**- Neg | Strong CW+ M | Strong CW+ M | Neg | Strong CW+ M | Neg | Moderate CW+M | Strong CW+ M |
| **2**- Neg | Neg | Mild M | Neg | Focal trace CW | Seg trace M | Neg | Neg |
| **3**- Neg | Seg trace CW | Strong CW | Neg | Trace CW | Seg trace CW | Trace CW | Trace CW |

**Conclusions:** It is tempting to speculate that a decrease of VEGF bioavailability in glomerular capillary walls has resulted in structural changes and proteinuria. We interpret the IgA deposits in one patient as a peculiar sign of tuft injury rather than an

IgA glomerulonephritis. Although currently rare, these glomerular lesions will likely be more prevalent with increased acceptance of anti VEGF therapy world wide.

### 1335 Idiopathic Nodular Glomerulosclerosis: Controversial Issues about Its Pathogenesis

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**Background:** Idiopathic nodular glomerulosclerosis (ING) is an enigmatic condition closely resembling diabetic nodular glomerulosclerosis without evidence of diabetic mellitus or other specific disease. ING remains a rare disease entity with an unclear pathogenesis. This study aims to evaluate the clinicopathologic features of 14 patients with ING.

**Design:** 14 cases of ING were identified in a retrospective review of renal biopsies between 1998 and 2006. Diabetic nodular glomerulosclerosis and other conditions with histological findings of nodular glomerulosclerosis were excluded by clinical, laboratory and histopathological investigations. Clinical data and laboratory results at the time of biopsy were analyzed in all cases. The renal biopsies were examined by light, immunofluorescence, and electron microscopy.

**Results:** The study cohort consisted predominantly of older (mean age 64.4 years) white (71%) women (64%). Clinical findings at the time of biopsy included: renal insufficiency (mean serum creatinine 2.8 mg/dl), proteinuria (mean 6.1 g/day), hypertension (93 %), history of smoking (64%) and obesity (BMI>30) (57%). Seven patients (50%) presented with nephrotic syndrome and one with preexisting extrarenal cardiovascular complications at the time of biopsy. Histopathological findings showed nodular glomerulosclerosis (100%), arterio-arteriolosclerosis (100%), prominent hyalinosis (71%) and moderate glomerular basement membrane thickening (86 %). IF and EM had no other specific findings.

**Conclusions:** Our results are consistent with the conclusion of others that ING is a clinico-pathological entity, closely associated with Caucasian ethnicity, advanced age and hypertension. The overall incidence of smoking in our study is lower than those reported in similar studies in the published literature. The slightly higher female proportion in our study group indicates that male sex hormones may not play a significant role in ING as previously suggested. Our data also shows that ING is closely associated with obesity. Further studies are needed to elucidate the pathogenesis of ING.

### 1336 Superoxide Dismutase Mimetic Drug, Tempol, Aggravates Anti-GBM Antibody-Induced Glomerulonephritis in Mice

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**Background:** Oxidative stress plays an important role in the pathogenesis of anti-GBM antibody-induced glomerulonephritis (GN). Superoxide dismutase (SOD) is the first and most important line of defense against oxidative stress by converting superoxide to hydrogen peroxide. We investigated the effect of SOD-mimetic drug, Tempol, on anti-GBM GN in mice.

**Design:** 129/svJ mice were challenged with rabbit anti-mouse GBM nephrotoxic sera to induce GN and subsequently divided into two groups. The study group received Tempol (200 mg/kg/day, orally) whereas the control group received vehicle for 3 weeks. Routine histology, SOD activity, lipid hydroperoxides (LPO), potential antioxidant (PAO) activity, and immunohistochemistrical staining for neutrophils, macrophages, p65-NFkB and osteopontin were performed.

**Results:** Unexpectedly, Tempol administration exacerbated anti-GBM GN as evidenced by presence of severe crescentic glomerulonephritis with neutrophil and macrophage influx, significant increase in proteinuria, and accelerated mortality in the treated group. Tempol treatment raised SOD activity in serum and urine, but not renal tissue. Serum and renal tissue LPO and PAO were comparable between the two groups. Finally, treatment with Tempol resulted in marked up-regulations of p65-NFkB and osteopontin in the kidney.

**Conclusions:** Tempol does not ameliorate anti-GBM GN. Instead, it aggravates renal disease possibly due to increased production of hydrogen peroxide which is a potent NFkB activator. This supposition is supported by increases seen in p65-NFkB, osteopontin as well as neutrophil and macrophage influx in the kidneys of Tempol-treated mice.

### 1337 Inflammation in Areas of Interstitial Fibrosis and Tubular Atrophy Show a Significant Correlation with B Cell Associated Transcripts and Inferior Prognosis of Renal Allografts

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**Background:** According to the current Banff classification, infiltrates in cortical areas of interstitial fibrosis and tubular atrophy (IFTA) should not be considered for the diagnosis of rejection.

**Design:** In 130 renal allograft biopsies we assessed semi-quantitatively the % cortex with inflammation in areas with IFTA (i-IFTA). The extent of i-IFTA was correlated with established Banff criteria, gene expression revealed by microarrays, and allograft function.

**Results:** Extent of i-IFTA correlated significantly with 202 of the 54675 microarray probe sets ($r>0.4$, $p<0.0001$). 57% of the correlated transcripts were not annotated by previously described pathogenesis based transcript sets of renal allograft rejection. 22% of the probe sets were annotated as B cell associated transcripts, 9% as injury-and-repair, 6% as kidney transcripts, but only 4% as T cell associated transcripts. In contrast, of the 483 transcripts found to be significantly correlated with infiltrates in non-scarred cortex (i.e. Banff i-score), none were B cell associated while 34% were T cell associated. The B cell richness in i-IFTA was confirmed by immunohistochemistry. The extent of i-IFTA showed a significant negative correlation with renal allograft function three months after treatment (r-0.26, p 0.004).

**Conclusions:** Infiltrates in IFTA show an association with B cell transcripts and many not yet annotated transcripts, while infiltrates in non-scarred cortex are predominantly related to T cell associated transcripts. This suggests different immunopathologic processes during renal allograft rejection in these two morphological compartments. Since i-IFTA is related to an inferior prognosis, further elucidation of the role of B cells in IFTA is required.

### 1338 Refining the Histological Banff i-Score Criteria Using Microarray Data from Renal Allograft Biopsies

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**Background:** Histology is the "gold standard" for diagnosing renal allograft rejection. The Banff classification provides criteria and thresholds for diagnosing rejection that were empirically developed, but lack independent immunopathological validation. Interstitial infiltrates in areas of fibrosis and tubular atrophy (IFTA), as well as perivascular and nodular infiltrates are not considered for the Banff i-score because they are regarded as non-specific. The aim of this study was to examine this Banff rule using microarray based expression data.

**Design:** In 130 renal allograft biopsies the extent of inflammation in non-fibrotic areas, in IFTA, perivascular, and nodular infiltrates was semi-quantitatively assessed by histology. We correlated the extent of the different infiltrates with the expression of pathogenesis based transcript sets (PBTs) representing major biological processes during renal allograft rejection.

**Results:** Considering currently ignored types of inflammation, especially those in areas of IFTA, improved the correlation between histology and PBT-expression: table 1 (all correlations shown are significant at $p<0.0001$, Spearman correlation). Receiver operating curves employing diagnostic thresholds based on PBT expression levels revealed greater areas under the curves if all types of inflammation, especially those in areas of IFTA, were considered compared to assessing the i-score according to Banff criteria.

**Conclusions:** Regarding changes in the transcriptome as an independent measurement of the inflammatory burden revealed current Banff criteria for making the i-score to be potentially flawed. Assessment of inflammation related PBTs might be suitable to refine empirical scoring schemata in diagnostic histopathology of all inflammatory diseases.

| PBTs (gene sets) | Banff* | i-IFTA | nodular | perivascular | Banff + i-IFTA | Banff + all |
|---|---|---|---|---|---|---|
| T-cell associated | 0.534 | 0.284 | 0.298 | ns | 0.726 | 0.741 |
| γ-Interferon dependent | 0.532 | 0.258 | 0.241 | ns | 0.706 | 0.703 |
| Macrophage associated | 0.539 | 0.243 | 0.232 | ns | 0.689 | 0.689 |
| Kidney parenchyma associated | -0.296 | -0.199 | ns | ns | -0.539 | -0.536 |
| Injury and repair associated | 0.379 | 0.246 | ns | ns | 0.636 | 0.645 |
| B-cell associated | 0.309 | 0.458 | 0.380 | ns | 0.641 | 0.668 |

### 1339 Baseline Structural Parameters Are Different in Cadaver vs. Living Kidney Donors

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**Background:** The impact of baseline structural parameters on post-transplant graft outcome has been controversial. This controversy might be partially due to inattention to select appropriate control subjects. Our lab showed that glomerular volume and ultrastructural parameters are different in cadaver (CD) vs. living (LD) donors. The aim of this study was to compare cortical interstitial fractional volume [Vv(Int/C)] and % sclerotic glomeruli (%SG) in pretransplant biopsies (PTBX) of LD and CD of similar age and the effect of these parameters on immediate graft function.

**Design:** PTBX from 46 (29 F), age 39(19-56) y/o LD and 23 (9F), age 38 (16-66) y/o CD were studied by stereologic methods. CD and LD PTBX were performed in recipient one hour after transplantation. Vv(Int/C) and fractional volumes of, proximal [Vv(PT/C)], distal [Vv(DT/C)] and atrophic tubules [Vv(AT/C)] and periglomerular fibrosis [Vv(PGF/C)] were measured on systematically sampled images of PTBX. % sclerotic glomeruli (%SG) was determined on serial sections. Recipients' graft functions were categorized as immediate (IGF) vs. slow/delayed (DGF).

**Results:** The age of LD (46±11 y/o) was not different from CD (50±12 y/o) recipients. DGF was more common in CD vs. LD ($p=0.02$). Vv(Int/C) was greater ($p=0.003$) in CD (0.19±0.04) vs. LD (0.23±0.05). However, Vv(Int/C) was not different between IGF and DGF. %SG was greater ($p=0.02$) in CD (4.7±5.8) vs. LD (1.8±3.2). However, %SG although greater, was not statistically different in DGF (5.1±6.9) vs. IGF (2.5±4.2). %SG, but not Vv(Int/C), was correlated with donor's age ($r=0.32$, $p=0.009$). %SG and Vv(Int/C) were not correlated. Neither %SG nor Vv(Int/C) were different in male vs. female donors. %SG was correlated with Vv(DT/C) in CD ($r=0.60$, $p=0.003$), but not in LD. Multiple regression analysis ($r^2=0.21$, $p=0.04$) showed that donor type (CD vs. LD) was the only independent predictor of immediate graft function ($p=0.004$).

**Conclusions:** Renal cortical interstitial fractional volume and % sclerotic glomeruli are greater in cadaver vs. living donors. Neither of these parameters is related to immediate graft function. The differences might result from altered homodynamic milieu or different sampling condition in cadaver kidneys. These differences make cadaver kidneys less than ideal to be considered as control in renal transplant structural studies.

### 1340 Rat Maternal Food Restriction (MFR) Inhibits Mesenchymal to Epithelial Transformation (MET) during Nephrogenesis

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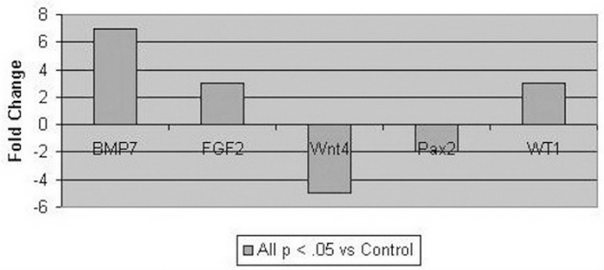
**Background:** In human and animal studies, maternal food restriction (MFR) leads to fetal and adult reduction in nephron endowment with hypertension and renal dysfunction in adults. Early, intermediate and late stages of nephrogenesis are regulated by different signaling mechanisms. We have shown MFR offspring to have 19% fewer glomeruli

and dysregulated TGFβ and IGF1 in early nephrogenesis. During intermediate nephron development, metanephric mesenchyme (MM) undergoes MET to form glomeruli and tubules. This process entails reciprocal regulation between Pax2 and WT1, both of which act as transcription factors for Wnt4, which drives MET. MET is inhibited by high concentrations of BMP7, which is maintained by FGF2 and inhibits apoptosis of MM. This study was performed to determine whether MET signaling is impacted by MFR thus participating in the nephropenia observed in MFR offspring.

**Design:** Rat dams were fed AdLib or a 50% MFR diet from embryonic day (e)10. Kidneys were removed from unsexed embryonic offspring at e20 (n=6 in each group). Total RNA was isolated and RT-PCR performed to assess mRNA levels of 5 key nephrogenic factors active in MET with 18S as the reference gene. Results are expressed as fold change relative to control.

**Results:** At e 20, all factors assessed were significantly dysregulated. There was upregulation of BMP7 (7-fold), FGF2 (3-fold), and WT1 (3-fold) with downregulation of Wnt4 (5-fold) and Pax 2 (2-fold) mRNA levels (all p< .05 vs controls).




**Conclusions:** At e20 when MET is an active part of nephrogenesis, Wnt4 is significantly reduced. The signaling leading up to and including the Wnt-4 pathway are critical components in MET and nephrogenesis. At the same time, FGF2 and BMP7 are upregulated, resulting in arrest of MET but promoting mesenchymal survival via reduction in apoptosis. WT1 and Pax2 regulate each other; therefore, it appears the overall effect of increased WT1 and decreased Pax2 mRNA levels is inhibition of Wnt4. Taken together, this represents a potential mechanism for reduced nephron endowment via interrupted MET occurring at this developmental stage in the MFR model of renal hypoplasia.

### 1341 Angiotensin II-Induced Tubulointerstitial Fibrosis in β6 -/- Mice Is Not Affected by AcSDKP Administration

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**Background:** αvβ6 integrin is an activator of transforming growth factor β (TGF-β). Mice deficient in β6 are resistant to unilateral ureteral obstruction (UUO)-induced tubulointerstitial fibrosis. We have previously shown that angiotensin II (AngII) treatment restores fibrosis in UUO-β6-/- mice without activation of TGF-β but with increased plasminogen activator inhibitor 1 (PAI-1), a molecule linked to fibrosis. Thymosin β4 (Tβ4), a G-actin sequestering protein with effects on angiogenesis, cell migration and matrix, is necessary for AngII induction of PAI-1 in cultured glomerular endothelial cells. We have previously shown that Tβ4 is a marker of early glomerulosclerosis and is increased when tubulointerstitial fibrosis is induced by UUO. Tβ4 is degraded to AcSDKP which has anti-fibrotic effects. We hypothesize that the balance of Tβ4-AcSDKP could modulate fibrosis in β6-/- mice. We therefore investigated whether the administration of AcSDKP via osmotic minipump has an effect on AngII restored fibrosis and Tβ4 expression in β6-/- mice.

**Design:** β6-/- or WT male mice (C57/Bl6 background) underwent UUO and were divided into two treatment groups: AngII (40μg/kgBwt/hr), AngII (40μg/kgBwt/hr) + AcSDKP (800 μg/kgBwt/day). Morphological investigations were performed at sacrifice at day 14 after UUO (n=7-8 each group). Masson/trichrome stained sections were scored to assess the percentage of injured tissue: 0= no injury; 1=<25%; 2=25-50%; 3=50-75% 4=>75%. Interstitial Tβ4 was scored as number of positive cells per hpf.

**Results:** Both groups had significant but similar injured tissue with tubular dilatation, atrophy and interstitial fibrosis (β6-/-UUO+AngII 2.19±0.26; β6-/-UUO+AngII+AcSDKP 2.10±0.12, p=NS). Thymosin β4 expression was also similar in mice with or without added AcSDKP (β6-/-UUO+AngII: 46.1±6.0; β6-/-UUO+AngII+AcSDKP 43.8±5.4 p=NS).

**Conclusions:** In β6-/- mice, AngII treatment superimposed on UUO restores interstitial fibrosis. This restored fibrosis is linked to increased Tβ4 staining. However, additional treatment with AcSDKP through osmotic minipump did not alter the level of injury nor did it influence the expression of Tβ4 in this model. We speculate that these findings could reflect an overdrive of AngII-mediated effects that are not dependent on PAI-1, and/or cell-specific differences in the Tβ4-AngII-PAI-1 axis in glomerular endothelial cells versus tubular/interstitial cells.

### 1342 Reproducibility Studies on Digital Pathology Slides Versus Glass Slides

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**Background:** With recent advancements in high resolution virtual microscopy technologies, it is now practical to create digital slides of an entire microscope slide with a superior quality and speed. Digitization of a glass slide has started to change the old fashion of Pathology practice not only in research and education, but also in clinical practice including digital slide archival, pathology read-outs, and consultations. We aimed to test the reliability of this new platform by comparing the reproducibility of histology using digital slides versus glass slides.

**Design:** Thirty-seven human renal allograft biopsies for cause (3 slides per each biopsy) were scanned with Aperio Slide Scanning System. The biopsies were scored according to Banff schema by 2 renal pathologists using both digital and glass slides. The scoring included 11 Banff lesions, and kappa statistics were used to assess the intra- and inter-observer reproducibility.

**Results:** Intraobserver reproducibility for Banff lesions using digital slides vs. glass slides was between moderate and almost perfect with a kappa value of 0.74 for overall diagnosis. Similar kappa values were obtained when glass slides or digital slides (0.77 vs. 0.93 for overall diagnosis, respectively) were read twice by an observer. Inter-observer reproducibility of digital slides was comparable to glass slides with kappa values of Banff lesions ranging between 0.10 and 0.86.

**Conclusions:** We conclude that the intra- and inter-observer reproducibility of digital slides are similar to of glass slides. Therefore, digital slides can easily be used in daily practice for diagnostic purposes, and would increase the availability of central pathology reading which is crucial for multi-center trials.

### 1343 External Validation of the Existing Banff Criteria for Rejection by the Transcriptomics

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**Background:** The weaknesses of the Banff schema are the absence of an independent external standard to test the classification; and its excessive reliance on histologic scoring, which has inherent limitations in reproducibility, particularly at the interface between borderline and rejection. The opportunity lies in new technology such as transcriptomics, which can form an external standard to test and improve the histologic criteria to reduce the non-specificity.

**Design:** We previously defined gene sets that reflect major biologic events in kidney allograft rejection and constructed a classifier that computes the probability of rejection (0-100%).

**Results:** By multivariate analysis, acute lesions (g, i, t, v) were independently associated with increased cytotoxic T cell transcripts whereas C4d, g, i, and t were related to Ifng effects ($R^2$=.55). Depression of parenchymal genes was related to i score ($R^2$=.26). Here we used a classification tree analysis (SPSS 15.0) using Banff lesions and C4d staining to investigate the best combinations of histologic lesions that predict probability of rejection defined by transcriptomics in 135 renal allograft biopsies for cause. This analysis showed the i score (interstitial inflammation) as the strongest predictor of rejection, which is followed by the g score (glomerulitis) (Figure 1).

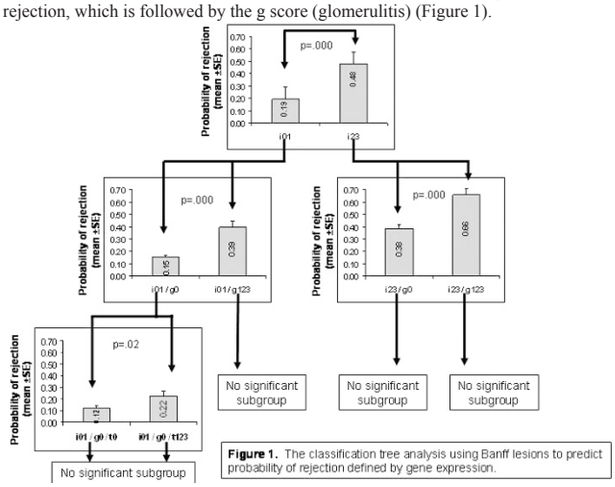


**Figure 1.** The classification tree analysis using Banff lesions to predict probability of rejection defined by gene expression.

**Conclusions:** At any given degree of i score, the probability of rejection was significantly higher if there is glomerulitis. Surprisingly, the power of glomerulitis (g), as a predictor of rejection, was much greater than tubulitis (t) or vasculitis (v), the lesions known as pathognomonic for rejection. The transcriptomics offers opportunities for objective, quantitative, biology-based measurements that will improve the histologic criteria to create an optimized gold standard.

### 1344 PAX-2 and Tamm-Horsfall Immunohistochemistry Facilitate the Diagnosis of Glomerular Cysts in Bilateral Cystic Kidneys

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**Background:** Glomerular cysts may be seen in hereditary cystic kidneys such as ARPKD/ADPKD, autosomal dominant glomerulocystic kidney disease (ADGCKD), and sporadic or syndromic renal dysplasia. A Glomerulocystic kidney is defined as containing a minimum of 5% glomerular cysts. Any of the above entities may present as a glomerulocystic. Glomerular cysts may be misidentified as tubular cysts when the glomerular tuft is not present in the plane of section resulting in misdiagnosis. The aim of this study was to evaluate specific nephron segment antibodies and lectins to identify glomerular cysts.

**Design:** The pathology archives at Washington University in St. Louis were searched for bilateral cystic kidneys and limited to pediatric patients (age <2) with tissue available. The slides were reviewed and a representative formalin-fixed, paraffin block was selected for staining using antibodies against Tamm-Horsfall protein (THP) and Paired box gene 2 (PAX-2) and Lotus tetragonolobus agglutinin (LTA) lectin. The stains identify distal tubules/collecting ducts, parietal epithelial cells/distal tubules,

and proximal tubules respectively. Immunohistochemical staining of the cysts were graded semi-quantitatively on a four point scale (1 = <10%, 2 = 11-25%, 3 = 26-50%, 4 = >50% positive cyst staining).
**Results:** A total of 22 cases were identified with glomerular cysts varying between 5% to >90% of glomeruli. Immunohistochemistry with PAX-2 was 3+ or greater in 20 of 22 cases. THP was 3+ to 4+ in only 2 cases. LTA ranged between 1+ and 3+ in all cases. The combination of high PAX-2 and low THP identified glomerular cysts. The data was analyzed with a forward stepwise multivariable regression and PAX-2 and THP were significant predictors of diagnosis (p=0.0144 and p=0.0005 respectively). LTA was not significant (p=0.1270). Using this panel, the majority of cases were identified as bilateral renal dysplasia 68% (15/22), 14% (3/22) were ARPKD, and 18% (4/22) were designated as glomerulocystic kidney. One case originally diagnosed as renal dysplasia associated with Perlman's syndrome was reclassified as glomerulocystic kidney.
**Conclusions:** A major problem in evaluating cystic kidneys is missing the glomerular cysts and misdiagnosing potentially hereditary kidney disease as sporadic renal dysplasia. A combination of high PAX-2 and low THP highlights the predominance of glomerular cysts and facilitates the diagnosis of glomerulocystic kidney.

### 1345 Clinicopathologic & Proteomic Assessment of Dense Deposit Disease Associated with Monoclonal Gammopathy of Undetermined Significance

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**Background:** Dense deposit disease (DDD) is a rare condition usually affecting children and young adults. Glomerular changes are not constant by light microscopy (LM), but often show a membranoproliferative pattern. Direct immunofluorescence (IF) often displays C3 staining of the glomerular capillary walls and mesangium. The diagnosis of dense deposit disease is based on the identification of characteristic intramembranous electron dense deposits by electron microscopy (EM). We describe six cases of a unique subset of older adult patients presenting with renal disease and a concomitant diagnosis of monoclonal gammopathy of undetermined significance (MGUS) that were diagnosed with DDD based on EM findings.
**Design:** We identified patients ≥55 years old diagnosed with DDD on kidney biopsy at Mayo Clinic since 1995. The clinical, LM, IF, and EM features were studied and compared. Laser microdissection was performed to remove affected glomeruli which were analyzed by gas chromatography mass spectroscopy (GCMS) for peptide content. Results were compared to tubules from the patient and normal controls.
**Results:** We identified nine patients ≥55 years of age diagnosed with DDD since 1995, of which, six (67%) had MGUS. Clinical, LM, IF and EM findings for each case are shown in the table.

| Case | Age/sex | Clinical | LM | IF | F/U |
|---|---|---|---|---|---|
| 1 | 77/F | Hematuria, IgG- Kappa | MPGN | C3 3+ | ESRD |
| 2 | 58/F | Nephrotic syndrome, IgG-Lambda | MPGN | IgG, IgM,& C1q 1+; C3 3+ | ESRD; recurrence in allograft & graft failure |
| 3 | 63/F | Proteinuria, IgG-Kappa | MPGN | IgG 2+; IgM 1+; C3 3+ | ESRD |
| 4 | 60/M | Proteinuria, IgG-Lambda | Diabetic glomerulopathy | C3 2+ | ESRD; myeloma at 120 months |
| 5 | 60/F | Proteinuria, IgG-Kappa | Mesangio-proliferative | C3 1+ | CRF |
| 6 | 74/M | Proteinuria, IgG-Lambda | Mesangio-proliferative | C3 1+ | CRF |

Proteomic assessment by GCMS showed a significant amount of complement precursor proteins C3, C5, and C9; Apolipoprotein E; complement factor H precursor protein and IgG constant chains in diseased glomeruli. These proteins were not detected in normal control glomeruli. Immunoglobulin light chains were not a significant proportion of the protein content.
**Conclusions:** DDD in patients with MGUS appears to be a distinct clinicopathologic entity that affects older patients, results in a poor clinical outcome, shows a variable histologic picture and consistently displays EM findings of DDD. GCMS analysis suggests that the deposits in DDD associated with MGUS are comprised of complement components and not monoclonal immunoglobulin light chains.

### 1346 Curcumin Enhaced the Replacement of the Glomerulopathic Light Chains Induced Apoptotic Rat Mesangial Cells by Rat Mesenchymal Stem Cell

*J Teng, Y Zhang, EA Turbat-Herrera, GA Herrera.* St. Louis University Medical Center, St. Louis, MO.
**Background:** In previous studies it has been shown that G-LCs (associated with both light chain deposition disease and AL-amyloidosis) cause apoptosis of mesangial cells. Curcumin has been demonstrated to increase apoptosis through activation of nF-kB. In this study, rat mesenchymal stem cells co-cultured with rat mesangial cells were incubated with G-LCs together with Curcumin to visualize the interactions among these cells as apoptosis was enhanced.
**Design:** Fisher 344 rat mesenchymal stem cell kit and rat mesangial cells isolated from Fisher 344 rat kidney (8-10 weeks old) were used for the experiments. PKH fluorescent cell linker kit was used to label the rat mesenchymal cells to distinguish them from the mesangial cells. Mesangial and mesenchymal stem cells were co-cultured and incubated with G-LCs. An Olympus IX81 living cell imaging system was used to observe the cell interactions every 10 minutes and to capture images. ApoTarget Annexin-V-FITC Apoptosis kit was used to detect apoptosis in mesangial and mesenchymal cells.
**Results:** G-LCs induced apoptosis in mesangial cells immediately following nF-kB activation and peaked at 3 days. G-LCs also induced apoptosis in mesenchymal stem cells following a similar time course. Curcumin enhanced the apoptotic effect of G-LCs. As cells became apoptotic, the ratio of labeled mesenchymal stem cells to mesangial cells increased. Eventually the mesenchymal stem cells replaced almost entirely the mesangial cells after treatment with G-LCs for 72 hours.
**Conclusions:** Although both mesangial and mesenchymal stem cells undergo apoptosis under the influence of G-LCs and Curcumin, mesangial cells were much more susceptible to apoptosis. Mesenchymal stem cells proliferated when mesangial cells underwent apoptosis, replacing damaged mesangial cells. Mesenchymal stem cells can play an important role in the "healing" of the damaged mesangium by G-LCs.

### 1347 Determination of a Molecular Signature of Acute Renal Allograft Rejection Using Quantitative Real-Time RT-PCR of 45 Genes on a Low Density Array

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**Background:** The histopathological diagnosis of acute rejection in renal allograft biopsies remains difficult in many cases. Quantitative gene expression in these biopsies was studied in order to identify a molecular signature corresponding to acute rejection.
**Design:** RNA was extracted from archival frozen renal allograft tissue from 14 cases of acute rejection and 28 cases of non-rejection. Quantitative real-time RT-PCR analysis of 45 selected genes involved in apoptosis, cell adhesion, cell signalling and growth, hypoxia response and T-cell activation was performed on a low density array and their levels of expression in cases of acute rejection and non-rejection were compared.
**Results:** Successful RNA amplification was achieved in 9 cases of rejection and 15 cases of non-rejection. At least threefold increased median expression of ICAM-1, αE-integrin and CXCL10 and at least threefold decreased median expression of Epidermal Growth Factor (EGF), bak-1 and hrk was seen in the rejection group as compared to the non-rejection group. EGF showed the largest difference in expression (over 6-fold decreased median expression in the rejection group) and was significant for discriminating between rejection and non-rejection cases in allograft tissue by $X^2$ analysis ($X^2$=13.2). The level of EGF expression was predictive of rejection with an accuracy of 83% as shown by 10-fold cross-validation analysis. Overexpression of genes of cytokines related to T-cell activation and underexpression of DARC, EGFR and bcl-w were also seen in the rejection group. Unsupervised hierarchical clustering of the cases based on the expression of 7 genes expressed in over 90% of cases (EGF, VCAM-1, E-cadherin, IL-18, sp1, TGFβ and osteopontin) resulted in separation of the rejection cases from all but 3 of the non-rejection cases.
**Conclusions:** Quantitative real-time RT-PCR analysis of a panel of multiple genes on low density arrays potentially allows the identification of a molecular signature of acute renal allograft rejection. Rejection and non-rejection cases could be largely separated by the expression levels of a panel of 7 genes, although only one of these (EGF) had expression levels that on their own were predictive of rejection.

### 1348 Glomerular Fibrin Thrombi in Early Renal Allograft Biopsies

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**Background:** In renal allograft biopsies, fibrin thrombi are associated with antibody mediated humoral rejection (AHR), thrombotic microangiopathy (TMA, often cyclosporine inhibitor toxicity, CNIT), ischemic necrosis, malignant hypertension, or sepsis. Glomerular fibrin thrombi (gFT) may be the earliest and only histologic indication of AHR in ABO-incompatible grafts (Fidler. AJT 4:101-7). We characterized early renal allograft biopsies from patients with blood group-identical allografts showing glomerular fibrin thrombi.
**Design:** The computerized renal pathology files of Oregon Health & Science University (2001-7) were searched for gFT in initial allograft biopsies; suspected surgical complications were excluded. Histopathologic findings (Banff criteria: glomerulitis, tubulitis, interstitial inflammation, arteritis, peritubular capillary (PTC) inflammation or dilation, C4d immunofluorescence staining) were correlated with clinical data, treatment, and renal outcome.
**Results:** Eleven biopsies were identified, performed a mean of 6.9 days post transplant (range 0-22 days); 3 were surveillance biopsies in highly sensitized patients. In 6/11 biopsies, gFT was the only light microscopic finding. 2/11 showed gFT with other features of AHR including glomerulitis, PTC dilation with neutrophils and mononuclear cells. 3/11 had concomitant vascular rejection (1-Banff 2B; 2-Banff 3 with arterial fibrinoid necrosis). Only 2/10 tested cases were positive for PTC C4d (both gFT-only histology).

| Histology | N | C4d+ PTC | F/up months | F/up creatinine mg/dL |
|---|---|---|---|---|
| gFT-only | 6 | 2/5 | 20 (range 3-44; n=5) | 2.0 (range 1.1-3.7; n=5) |
| gFT-AHR | 2 | 0/2 | 16 | 0.9 (n=1) |
| gFT-Banff 2 | 1 | 0/1 | 12 | 1.3 (n=1) |
| gFT-Banff 3 | 2 | 0/2 | 2 | 1.7 (n=1; other graft lost) |

The majority of patients were treated with IVIg and plasmapheresis (+Thymoglobulin (TG) or rituximab in 2 cases). gFT-Banff 2 rejection was treated with TG alone; one patient was also switched to sirolimus (presumed TMA/CNIT). At a mean of 16 months post transplantation (range 2-44); 8 patients had a mean sCr of 1.7 mg/dL (range 0.9-3.7); one allograft was lost (Banff 3 rejection).
**Conclusions:** This series illustrates that gFT may be the only initial histopathologic manifestation of AHR in blood group-compatible allograft biopsies. gFT may appear before detectable C4d, as in ABO-incompatible allografts. Recognition of this pattern in early allograft biopsies, in correlation with CNI drug levels and perfusion studies, is critical in guiding effective treatment.

### 1349 Lymphangiogenesis in Chronic Obstructive Uropathy

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**Background:** Chronic tubulointerstitial injury is a constant feature of urinary obstruction (UO), but its pathogenesis is not completely understood. The role of lymphatics in the renal injury including that associated with UO has not been elucidated. They may involve clearance of interstitial fluid or channeling kidney-specific antigens to lymphoid organs.

**Design:** Consecutive tissue sections of kidneys with UO (15) and normal kidneys (6, from "normal" areas of nephrectomy specimens for UO or tumor, or donors' kidney) were subjected to immunostain for D2-40 (a specific marker for lymphatic endothelial cells), T cells (CD3), B cells (CD20), macrophages (CD58), vascular endothelial growth factor (VEGF) C and D (mitogens for lymphatic endothelial cells). The findings were quantified and correlated with morphologic changes.

**Results:** In normal kidneys, lymphatic vessels were rare in cortex (mean 0.05 /HPF), usually accompanied small arteries, but were not seen in medulla. Kidneys with UO showed numerous lymphatics (mean 3.4/HPF, a 60-fold increase) seen throughout areas of injury in both cortex and medulla. Early lesions of UO showed patchy but marked interstitial inflammation, in which lymphangiogenesis was limited to areas of inflammation, and was associated with T cell areas, but not seen within the B cell aggregates. Late lesion was characterized by severe tubular atrophy and interstitial fibrosis with little inflammation, but the lymphatic densities were similar to those of the early lesion. Both VEGFs C and C were focally noted in the lymphoid cells in areas with lymphangiogenesis, but not in tubular epithelial cells.

**Conclusions:** Marked lymphangiogenesis is a constant feature of the renal injury associated with UO. This is closely associated with lymphoid cell infiltration, and may be in part mediated by VEGF C and/or D from these cells. Newly formed lymphatics with a distinctive distribution may imply an element of immune-mediated mechanism for UO-mediated renal injury, and their persistence even after lymphoid cell clearance may imply a role for the progression of these lesions.

### 1350 Renal Injury Associated with Urinary Obstruction. Participation of an Immune Mechanism?

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**Background:** Chronic renal tubulointerstitial injury is a constant feature of uninary obstruction, but its mechanism is not fully elucidated. In spite of marked inflammatory cell infiltrates in this condition, even without superimposing infection, their roles are not well studied.

**Design:** Consecutive tissue sections of kidneys with urinary obstruction (15) were subjected to immunostain for T cells (CD3), B cells (CD20), macrophages (CD58), plasma cells (CD138), dendritic reticulum cells (CD21), and MIB-1, a cell proliferation marker. The findings were semiquantified and correlated with morphologic changes

**Results:** Interstitial inflammation was a constant feature and was composed of two related but distinct components. The first was characterized by diffuse, mutifocal, often marked infiltration of T cells (#70%) and B cells (#30%) with a moderate proliferation rate, fewer macrophages, and an absence of dendritic reticulum cells. These changes were closely associated with abundant newly formed lymphatics. The second was characterized by nodular aggregations of inflammatory cells reminiscent of lymphoid follicles. They were composed almost entirely of actively proliferating B cells admixed with dendritic reticulum cells, surrounded by predominantly of T cells admixed with newly formed blood vessels displaying prominent endothelial cells, but without lymphatics. Few mature plasma cells were also noted. In early lesion both types of inflammation were noted. In late lesion, nodular type of inflammation persisted, whereas the diffuse inflammation was much attenuated.

**Conclusions:** The findings suggest the possible roles of inflammation in the pathogenesis of the renal injury associated with urinary obstruction through different mechanism. Whereas T cell infiltration may mediated direct injury, the neoformation of intrapenchymal lymphoid tissue may imply an *in situ* or distant immune-mediated mechanism that can perpetuate the injury beyond the early phase.

### 1351 Relative Incidence of Biopsy-Proven Glomerular Disease – Comparative Study – 1997 Versus 2007 – Hennepin County Medical Center, Minneapolis, Minnesota

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**Background:** We report and compare the relative incidence of biopsy-proven glomerular disease (GD) across a 10-year interval reviewed at Hennepin County Medical Center, Minneapolis, MN. Our referral base includes nephrologists from urban, as well as biopsies received from suburban and rural Minnesota.

**Design:** Patient characteristics (age, sex, creatinine, 24 hour protein excretion, and indication for biopsy) and GD were recorded on 348 consecutive native biopsies from 1997 and 362 consecutive native biopsies from 2007. Transplant biopsies and biopsies with a diagnosis of vascular or tubulointersitial processes were excluded. In 1997 and 2007, 276 and 271 renal biopsies, respectively, showed a pathologic glomerular process.

**Results:** The patient characteristics were similar for each sampled year including: age (average of 50.2 [18-88] in 1997 compared to 52.5 [18-90] in 2007), gender, serum creatinine (average of 3.3mg/dl in 1997 compared to 3.1 mg/dl in 2007), and percentage of patients with nephrotic range proteinuria (58.1% in 1997 compared to 55.5 % in 2007).

| Glomerular disease | 1997 | | 2007 | |
|---|---|---|---|---|
| | n | % | n | % |
| Diabetic glomerulosclerosis (DGS) | 25 | 9 | 41 | 15 |
| End Stage kidney disease (ESKD) | 17 | 6 | 22 | 8 |
| Focal and segmental glomerular sclerosis (FSGS) | 36 | 13 | 29 | 10 |
| IgA nephropathy (IgAN) | 59 | 21 | 41 | 15 |
| Lupus nephritis (LN) | 28 | 10 | 36 | 13 |
| Membranoproliferative glomerulonephritis (MPGN) | 13 | 4 | 7 | 2 |
| Membranous nephropathy (MGD) | 31 | 11 | 26 | 9 |
| Minimal change disease (MCD) | 3 | 8 | 18 | 6 |
| Necrotizing/cresentic glomerulonephritis (NGN) | 26 | 9 | 27 | 10 |
| *Other | 19 | 6 | 24 | 8 |

*Other glomerular processes, including; amyloidosis, fibrillary glomerulonephritis, glomerular basement membrane disorders, nondiagnostic specimens, and post-infectious glomerulonephritis.

**Conclusions:** In 1997, the most frequent pathologic pattern of GD was IgAN followed in decreasing order of frequency by FSGS, MGN, LN, DGS, MCD, NGN and MPGN. In 2007, the most frequent type of biopsy–proven GD is DGS and IgAN. The frequency pattern of remaining glomerular processes is similar to 1997. DGS increased the most over the 10-year period, corresponding with national trends. FSGS is identified more frequently than MGN, a trend identified in other biopsy studies in patients with nephrotic syndrome. Also, the relative incidence of IgAN decreased during this period. This is a large database and may reflect the demographics of renal disease throughout Minnesota.

### 1352 Associations of C4d Deposition, Transplant Glomerulopathy and Rejection in Renal Allograft Biopsies Performed 10 or More Years after Transplantation

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**Background:** Chronic allograft injury may be secondary to immunologic and nonimmunologic factors. There are limited reports of the pathologic findings in long term renal allografts, particularly on the significance of C4d deposition in peritubular capillaries (PTC). We reviewed the pathologic findings in long surviving renal allografts which developed acute or chronic dysfunction prompting biopsy.

**Design:** Among all renal allograft biopsies received at the University of Washington between April 2003 and August 2007 (n=1601) we searched for biopsies that were performed 10 or more years following transplantation and were examined by immunofluorescence microscopy or immunohistochemistry for expression of C4d in PTC. We recorded the presence of glomerular, tubulointerstitial, and vascular pathology.

**Results:** We identified 87 patients: 38 females and 49 males that underwent a total of 103 allograft biopsies at 10 to 37 years following transplantation (10-14y=71, 15-20y=26, 21-37y=6). The results are as follow:

| | C4d + | C4d - | p Value * |
|---|---|---|---|
| ACR + | 14 | 10 | p < 0.01 |
| ACR - | 8 | 55 | |
| AVR + | 5 | 2 | p < 0.01 |
| AVR - | 17 | 63 | |
| TG + | 15 | 8 | p < 0.01 |
| TG - | 7 | 57 | |
| IgAN recurrent | 1 | 2 | - |
| IgAN de novo | - | 2 | - |
| DM recurrent | - | 5 | - |
| DM de novo | - | 2 | - |
| LN recurrent | 1 | 1 | - |
| MGN de novo | 1 | - | - |

ACR: acute cellular rejection; AVR: acute vascular rejection; TG: transplant glomerulopathy; IgAN: IgA nephropathy; DM: diabetic nephropathy; LN: lupus nephritis; MGN: membranous glomerulonephritis.

* p - value calculated by chi-square test. Ten (11%) patients had arteriolopathy characteristic of calcineurin inhibitor toxicity. The remaining 34 (39%) patients demonstrated various degrees of global glomerulosclerosis and associated interstitial fibrosis and tubular atrophy without any identifiable specific etiology. None of the patients had features of BK polyomavirus infection.

**Conclusions:** C4d staining of PTC is present in a substantial subset (25% in this series) of renal allograft biopsies performed 10 or more years following transplantation and may be seen as long as 24 years after transplantation. The presence of C4d staining in these allografts correlated significantly with TG and ACR. AVR was infrequent, but when present it correlated significantly with C4d positivity and TG.

### 1353 C4d Deposition and Vasculopathy in Renal Explants Following Chronic Rejection

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**Background:** Currently, the deposition of complement factor C4d in peritubular capillaries is seen as a reliable marker of antibody mediated renal allograft rejection. C4d is a decomposition product of complement factor C4, a member of the classical and mannan binding lectin complement pathways. Upon complement activation by antibody, C4d retains a covalent linkage to the target tissue thus making it a reliable and relatively stable hallmark of humoral rejection. Several studies have correlated C4d deposition with morphological changes and clinical outcomes using renal allograft biopsies, but little work has been done utilizing tissue obtained from allograft nephrectomies (ANs).

**Design:** Tissue derived from 15 ANs with late graft failure (graft survival >12 mo.) was evaluated for vascular changes. Concomitant glomerular and tubulointerstitial changes were scored using the Banff scoring scheme. C4d immunohistochemistry was performed on paraffin embedded sections utilizing an immunoperoxidase visualization

system. In addition, a subgroup of these patients were screened for antibody to major and minor histocompatibility antigens with solid phase assays using purified antigens and the Luminex technology.

**Results:** Thirteen of fifteen (13/15) explants were found to be positive for C4d deposition indicating a humoral component of rejection. C4d deposition was also found to strongly correlate with the presence of vascular fibrinoid necrosis, but no other correlation with evaluated vascular changes could be noted. Nearly all cases demonstrated moderate or severe tubular atrophy, glomerulosclerosis, interstitial inflammation, and vascular intimal sclerosis. All recipients had detectable circulating antibodies against both major (HLA Class I and /or II) and/or Minor Histocompatibility Antigens. Notably, the antibodies present were donor specific in the majority of recipients tested.

**Conclusions:** The results of this study confirm the substantial role of humoral rejection in chronically rejected renal allografts and provides further support for the correlation between C4d deposition and fibrinoid necrosis previously reported in other studies of acute humoral rejection utilizing renal allograft biopsies. The most striking observation of this study was severe vascular intimal sclerosis of large arteries in a preponderance of cases. This finding in C4d positive cases lends further support to studies performed in animal models that suggest a causative role of antibody mediated mechanisms in transplant vasculopathies.

### 1354 Amelioration of Progressive Renal Injury by Peroxisome Proliferator-Activated Receptor-gamma (PPARγ) Agonist in Aging Rats

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**Background:** We have previously shown that PPAR agonist protects against disease-associated glomerulo-sclerosis. Short-term treatment with PPARγ agonist also reduces inflammation in aging rat. The aim of the present study was to investigate whether long-term treatment with pioglitazone, a PPARγ agonist, could protect against sclerotic renal injury in aging.

**Design:** 14-month-old male SD rats (Zivic Miller) were given 10mg/kgBW of pioglitazone (Pio) for 6 months with normal diet as control (Cont). Urine protein and glomerular filtration rate (GFR) were measured. Kidneys were harvested for morphologic assessment at 20 months of age.

**Results:** Aging significantly increased urine protein compared with baseline (648.50±79.52 *vs*. 51.59±12.80 mg/24hr, P<0.05), while piogitazone decreased proteinuria in aging rats (227.56±71.63 mg/24hr, P<0.05 *vs.* Cont). At month 20, GFR was decreased to 0.38±0.09 ml/min in Cont, while Pio protected against this decline (0.83±0.16 ml/min, P<0.05). Severe interstitial fibrosis, tubular atrophy and glomerular sclerosis were found in aging kidneys. Pio treatment decreased glomerular sclerosis index (1.41±0.36 *vs.* Cont 2.28±0.21, P<0.05), but did not improve the vascular necrosis in kidney. 4-hydroxy-2-nonenal, a marker of lipid peroxidation which was not detectable in the kidneys at baseline, was expressed in tubular and glomerular cells at month 20, and was significantly inhibited by Pio (Cont 44.50±6.56 *vs.* Pio 27.69±3.26 positive cells/hpf, P<0.05).

**Conclusions:** We conclude that treatment with PPARγ agonists protects against renal injury in aging model, which is partly related to reduction of oxidative stress.

### 1355 Synergistic Effects of AcSDKP and Angiotensin Receptor Blocker on Repressing Plasminogen Activator Inhibitor-1 (PAI-1) Expression in Early Renal Interstitial Fibrosis

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**Background:** Our previous study shows that the G-actin sequestering protein thymosin β4 (Tβ4) is increased in the unilateral ureteral obstruction (UUO) model of tubulointerstitial fibrosis. We now investigated the effects and mechanisms of AcSDKP, an antifibrotic degradation product of Tβ4 which is further inactivated by angiotensin-converting enzyme (ACE), on early stage of UUO.

**Design:** Male C57BL/6 mice were sacrificed at day5 after UUO and treatments: control without treatment, AcSDKP (800μg/kg Bwt/day, s.c.), angiotensin receptor blocker (ARB) losartan (80mg/L) or AcSDKP+ARB.

**Results:** By immunohistochemistry, Tβ4 immunostaining was increased compared to normal in the interstitium, tubular cells, and glomeruli (mostly podocytes). Combination treatment dramatically decreased Tβ4 expression (Tβ4-positive interstitial cells 4.0±1.0 combination vs 12.3±1.8/HPF control; Tβ4-positive tubule profiles 0.3±0.2 combination vs 1.5±0.2/HPF control; and Tβ4-positive podocytes 1.9±0.4 combination vs 4.0±0.5/glomerulus control, all p<0.05 vs control). However, Tβ4 expression was not decreased by AcSDKP or ARB alone (Tβ4-positive interstitial cells: AcSDKP 11.5±1.3/HPF; ARB 25.5±4.9/HPF; Tβ4-positive tubule profiles: AcSDKP 1.3±0.4/HPF; ARB 2.1±0.2/HPF; and Tβ4-positive podocytes: AcSDKP 3.6±0.5/glomerulus; ARB 3.5±0.1/glomerulus). PAI-1 expression by western blot was significantly suppressed by combination treatment (0.53±0.10 combination vs 0.74±0.05 control, p<0.05), while AcSDKP or ARB alone did not affect expression (AcSDKP 0.59±0.05; ARB 0.69±0.12). There was no difference in fibroblast-specific protein-1 (FSP-1), a possible marker of epithelial-mesenchymal transition (EMT), among these four groups (Control 0.60±0.05; AcSDKP 0.57±0.05; ARB 0.51±0.01; combination 0.51±0.07).

**Conclusions:** We conclude that AcSDKP and ARB have synergistic effects on repressing the expression of PAI-1 and thymosin β4, but not on EMT. We speculate that thymosin β4 may be a novel target for modulation of interstitial fibrosis.

# Liver & Pancreas

### 1356 Adequacy of Liver Biopsies: Variability across Different Methods of Specimen Acquisition

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**Background:** Histological assessment of the liver is considered the gold standard for confirmation, diagnosis and assessment of severity of disease. Percutaneous liver biopsy (PLB) and transjugular liver biopsy (TJLB) are the main modalities to accomplish this. From a diagnostic perspective, a minimum specimen length of 15mm and the presence of at least 6 portal tracts (PT) are deemed usually adequate for confident histologic assessment. Our aim was to compare adequacy of PLB (performed either blind or ultrasound (US)-guided) and TJLB specimens for the diagnosis and staging of liver disease.

**Design:** METHODS: We evaluated 227 consecutive liver biopsy specimens (transplants and mass biopsies excluded) performed either blind percutaneous by hepatologists using a 15G Jamshidi needle (N=101), US-guided percutaneous by radiologists using an 18G automated cutting needle (N=108), or transjugular using an 18G Quickcore needle (N=18). Differences in specimen adequacy as judged from sample length, width, fragmentation, as well as number of evaluable PTs were sought.

**Results:** Blind percutaneous biopsies were significantly longer and wider (median (range) length 23 (6-46) mm and width 0.96 (0.09-1.18) mm) than US-guided percutaneous biopsies or TJLB (p=0.000, p=0.001 respectively). The median (range) of total number of PTs in the blind percutaneous biopsy group was 16 (2-47), which was superior to both the US-guided and TJLB groups (p=0.010, p=0.013 respectively). The median (range) of complete PTs (defined as the presence of >3/4 of PT circumference) in the blind percutaneous group was 11 (0-31), also superior to TJLB group (p=0.118). No differences in core length and number of PTs were noted when comparing US-guided and TJLB groups alone (p=0.857, p=0.424 respectively). Blind percutaneous biopsies were more often judged adequate than US-guided and TJLB specimens when applied to a more liberal (> or =15mm length AND > or =6 PTs) or stringent (> or = 20mm length AND > or =11 PTs) adequacy criteria (p=0.000 and p=0.001 respectively). The presence of established cirrhosis and left liver lobe biopsy site were associated with lower specimen widths (p=0.005, p=0.002 respectively). Also, specimen length and width were both positively correlated with number of complete PTs.

**Conclusions:** Liver biopsies performed blind percutaneously by hepatologists yield more adequate specimens when compared to US-guided or transjugular approaches. Differences in technique and diagnostic awareness may account for the observed trend.

### 1357 Biliary Intraepithelial Neoplasia (BilIN): Prevalence and Differences among Liver Explants for Alcoholic Cirrhosis, Hepatitis C Infection, and Non-Cirrhotics

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**Background:** Biliary intraepithelial neoplasia (BilIN) encompasses a spectrum of atypical or proliferative lesions of the large intrahepatic bile ducts. It is believed to be one pathway leading to the development of intrahepatic cholangiocarcinoma (CCA) through a dysplasia - carcinoma sequence. Recently, a large interobserver agreement study in patients with hepatolithiasis and PSC has proposed diagnostic criteria for three categories of BilIN based on increasing grades of nuclear atypia and loss of polarity: BilIN-1, BilIN-2, and BilIN-3 (*Mod Pathol* 2007;20:701-9). BilIN has not been systematically studied as a potential CCA precursor in patients with nonbiliary liver disease.

**Design:** We submitted 12 paraffin blocks targeted to the large intrahepatic/hilar ducts of 244 livers explanted for alcoholic (EtOH) cirrhosis (n=94), hepatitis C (HCV) cirrhosis (n=44), EtOH+HCV (n=26), and noncirrhotics (e.g., massive hepatic necrosis) (n=80). The resulting H&E sections were jointly reviewed by 2 authors and all bile ducts were classified as normal, reactive, or BilIN-1, 2, or 3. BilIN was further categorized as flat or (micro) papillary and was quantitated in each explant.

**Results:** Livers transplanted for EtOH and EtOH+HCV cirrhosis had the highest prevalence of BilIN, greater numbers of ducts with BilIN, and a shift toward higher grades of BilIN as compared to HCV alone and to noncirrhotics. Papillary or micropapillary forms of BilIN were also more common in EtOH cirrhosis than in other groups. BilIN-3 was seen only in the setting of cirrhosis (8 of 164, 4.9%) and was associated with CCA (2 cases) or mixed hepatocellular/CCA (1 case) elsewhere in the liver.

| | Highest Grade of BilIN | | | | Other Characteristics | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | Papillary BilIN (any grade) | ≥10 Foci BilIN (any grade) |
| **EtOH Cirrhosis** | 3.2% | 35.1% | 57.4% | 4.3% | 46.8% | 91.5% |
| **EtOH+HCV** | 3.8% | 38.5% | 53.8% | 3.8% | 19.2% | 92.3% |
| **HCV Cirrhosis** | 18.2% | 54.5% | 20.5% | 6.8% | 22.7% | 61.4% |
| **Noncirrhotics** | 45% | 38.7% | 16.3% | 0% | 17.5% | 33.8% |
| p value (ANOVA) | <0.0001 | | | | <0.0001 | <0.0001 |

**Conclusions:** When sectioning is directed toward the large intrahepatic bile ducts, low grade BilIN (BilIN-1,2) is a common finding in EtOH and EtOH+HCV cirrhosis, followed in statistical order by HCV cirrhosis and noncirrhotic liver disease. In contrast, BilIN-3 is infrequent and essentially restricted to cirrhosis. These findings suggest a potential role for alcohol and HCV in the development of CCA.