Design: The Eastern Division of the Cooperative Human Tissue Network (CHTN), an NCI sponsored prospective tissue procurement organization, has developed a programmatic informatics approach to tracking human biospecimens and data, while guaranteeing confidentiality under HIPPA. The informatic application assigns a unique identifier to each aliquot, insuring compliance with recommended Best Practices of ISBER, NCI and AATB.
Results: This set-theory approach is one of grouping individual aliquots into specialized entities for tracking and processing purposes. Each aliquot is given a unique identifier. This unique identifier is then referentially linked to one or all of the following group entities: 1) Patient Health Information Entity: denotes information about the donor. Information is kept on a secured data warehouse behind layered firewalls in an encrypted format. 2) Biosample Entity: tracks histopathologic characteristics of all the aliquots within entity. 3) Slide/Fluid Entity: references multiple quantities of individual aliquots allowing precise inventory tracking, while permitting processing of all entity member aliquots together. 4) Associative Entity: marks aliquots received from a single procedure. 5) Distribution Entity: relates all aliquots that are assigned for distribution to a single investigator. 6) Information Entity: stores digitized documentation in a secure environment, such as pathology reports, chart reviews, informed consent documents and/or slide images which pertain to an aliquot or a group of aliquots.
Conclusions: The developed application solves the question of individual aliquot labeling while preserving correlation with histopathology review procedures that guarantee proper fulfillment of investigator requests for human biosamples and data, inventory tracking, patient confidentiality, and content management documents.

1502 Use of Cloud Computing with Whole Slide Imaging for Interobserver Concordance Study

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Background: Pathologists routinely use glass slide concordance studies to assess interobserver agreement for specific diagnoses upon which diagnostic criteria, treatment and outcome are predicated upon. These types of studies require glass slides or necessitate pathologist travel for "multi-headed" conferences. The use of cloud computing technologies with whole slide images (WSI) allows for participants worldwide the convenience of high-resolution images virtually anytime from anywhere negating the need for transportation of glass slides or time away from clinical duties. Treatment of patients with autoimmune pancreatitis (AIP) and its various forms is highly dependent on pathological evaluation. The use of WSI remote review was performed to assess interobserver agreement.
Design: Forty cases of AIP were selected from the files of 3 study pathologists. Examples of lymphoplasmacytic sclerosing pancreatitis, idiopathic ductal centric pancreatitis, alcoholic pancreatitis and chronic obstructive pancreatitis were provided to 12 pathologists worldwide in a shared image library from to familiarize participants with the morphologic features for each entity. The cases were randomly ordered within the online library on a shared server. Images were viewable from any web-enabled PC using participants' choice of Internet browser. Slides were scanned at a central location and hosted on a cloud computing platform at no cost to study participants.
Results: The study participants reported a high level of technical satisfaction with ease of use of the site as well as image quality. No diagnoses were deferred based on image quality. Initial difficulties with account management for individual notification were resolved in a timely fashion. Questionnaire data detailing the presence of absence of 20 distinct histologic features was reviewed in real-time as participants completed reviews. The overall diagnostic agreement for the correct diagnosis amongst the 4 diagnostic categories high and comparable to glass slide diagnosis.
Conclusions: Digital pathology applications such as WSI with cloud computing allows for remote review of high quality images to be shared quickly, easily and conveniently with independent pathologist review negating the need for unnecessary courier glass slide services distant travel. Slides do not have to be returned, nor are they likely to be lost or damaged. Workflow of review of cases can be balanced and interpreted in real-time with enhanced distributed peer review.

Kidney

1503 Proliferative Glomerulonephritis with Monoclonal IgG Deposits Recurs or May Develop De Novo in Renal Allografts

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Background: Proliferative glomerulonephritis with monoclonal IgG deposits (PGNmIgGD) is a recently recognized glomerular disease (JASN 20:2055, 2009). The glomerular deposits are mostly IgG3 kappa and, unlike in the usual forms of monoclonal immunoglobulin deposition disease, extraglomerular deposits do not occur. The light microscopy resembles membranoproliferative glomerulonephritis (MPGN) with variable degree of glomerular lobularity/nodularity. Recurrence or de novo PGNmIgGD in renal allografts has not been reported yet.
Design: We reviewed our renal biopsy files since 01/01/03 to identify patients with PGNmIgGD in native and transplant kidney biopsies. IgG subtype staining was performed in all biopsies by immunofluorescence on frozen sections.
Results: We identified 16 patients with PGNmIgGD (0.27% of our biopsies); 3 of them have been reported previously as part of a large series (JASN 20:2055, 2009). The morphology and clinical course was similar to the previous series. The patients were mostly Caucasian females (14), had IgG3 kappa deposits (14) and had a relatively slowly progressive renal disease with severe proteinuria. Only two patients had monoclonal IgG kappa spikes in the serum; no patient had myeloma. In 15 patients, the disease appeared in the native kidney. A 68-year-old female patient had de novo disease with glomerular IgG1 kappa deposits in a renal allograft 13 years post transplant (native kidney disease was polycystic kidney disease). The graft failed 16 months following the biopsy. One patient with glomerular IgG3 kappa deposits underwent renal transplantation three years after the diagnosis of PGNmIgGD in his native kidneys. He developed recurrent disease with similar glomerular IgG3 kappa deposits one year post transplant. The patient died of a heart attack 1 ½ year after the transplant biopsy. He had 8 to 13 g/24h proteinuria and serum creatinine levels between 1.5 and 2.0 mg/dl at and after the diagnosis of recurrent disease.
Conclusions: Ours is the first report describing the appearance of PGNmIgGD in renal allografts. The disease can be de novo or recurrent. The recurrent disease developed rapidly, causing heavy proteinuria. Although based on our series we cannot determine the risk of recurrence in an individual patient, it is probably high. Our cases provide further proof that PGNmIgGD is a distinct glomerular disease entity.

1504 Primary Focal Segmental GlomerulosIcerosis Pathologic Variants in Adults and Children

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Background: The Colombia classification proposed five histopathologic variants of idiopathic Focal Segmental GlomerulosIcerosis (FSGS), namely collapsing (COLL), cellular (CELL), tip lesion variant (TIP), not otherwise specified (NOS) and perihilar (PH). Their clinical and prognostic implications have been examined in several biopsy series of adult patients, and these studies have shown worse prognosis of COLL, and better for TIP. We examined whether similar correlations were present for these different pathologic variants and their clinical characteristics and prognosis in biopsies from our practice, including both adult and children.
Design: All biopsies diagnosed as primary FSGS by LM, IF and EM in our referral practice from 1995 till 2006 were reviewed. Diagnosis was based on the presence of at least one segmentally sclerotic glomerulus, negative IF and extensive foot process effacement (>70%) without specific evidence of a secondary etiology. Biopsies were then classified by the Colombia Schema. Clinical history was reviewed and follow-up was classified as complete (CR) or partial remission (PR) of proteinuria, no remission (NR) or end stage renal disease (ESRD).
Results: 168 patients (92 male,74 female) met entry criteria. Average age was 42 ± 21 years (range 2 to 85) with 27 patients ≤ 18 year. 42 (25%) cases were African American and 86 (51%) were Caucasian, with ethnic origin other or not specified in the remaining patients. The frequency of FSGS variants was 13.7 % (N=23) COLL, 8.3% (N=14) CELL, 21.4% (N=36) TIP, 51.8 % (N=87) NOS, 4.8 % (N=8) PH. Black race showed more COLL and NOS compared to other variants and less in TIP. Edema and nephrotic proteinuria were present more in COLL and TIP. Hypertension and high serum creatinine at presentation were seen more in COLL and NOS. Outcome analysis of available data showed NR and ESRD more in COLL and CELL, compared to NOS and TIP. Conversely, NOS and TIP lesions had more PR or CR than other types. NOS was most common in children, and showed similar outcomes as in adults.
Conclusions: Our data support that different histopathologic variants have different clinical presentation and also different prognosis, regardless of age of patients.

1505 Prospective Assessment of C4d Deposits on Circulating Cells, Glomeruli, and Peritubular Capillaries in Lupus Nephritis (LN)

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Background: Classical complement activation plays a key role in the pathogenesis of LN. Studies have suggested an association between C4d deposition in native renal tissue and LN severity. Cell-bound complement activation products have not been systematically studied in LN. We aimed to compare the C4d deposition on circulating cells and renal tissues between LN and non-systematic lupus erythematosus (SLE) renal disease controls and to determine the association of C4d deposition with LN class and disease activity.
Design: We prospectively evaluated 13 LN and 7 non-SLE renal control subjects who underwent renal biopsy. Concurrent C4d levels on circulating erythrocytes and platelets (EC4d, PC4d) were measured by flow cytometry. The distribution of immunoperoxidase C4d on formalin-fixed, paraffin-embedded renal biopsy tissues was semiquantitatively assessed (0-3; negative to diffuse) in peritubular capillaries (PTC) and glomeruli [GBM and/or mesangium]. LN histologic class, and activity (AI) and chronicity (CI) indices were assessed using the 2004 ISN/RPS classification and the NIH scoring systems, respectively.
Results: Six LN subjects (46%) had class IV [IV(S) (3) and IV(G) (3)]; the remainder had classes II (15%), III (23%) and/or V (38%). Median AI and CI were 6 and 2, respectively. Median EC4d levels of LN subjects were higher compared to controls (19.2 vs. 7.0, p=0.03). PC4d was detected in 6/13 (46%) LN subjects vs. 0/7 (0%) controls (p=0.05). EC4d level significantly correlated with LN AI (r=0.67, p=0.01). Furthermore, subjects with class IV LN had higher median EC4d levels compared to other classes (33.2 vs. 16.2, p=0.046). In renal tissues, mean glomerular C4d score was higher in LN (2.3+/-1.2) compared to controls (1.3+/-1.1, p=0.03), while PTC C4d score showed no significant difference. This C4d staining was associated with glomerular immune complex deposits. Neither glomerular nor PTC C4d score correlated with LN class or AI. Neither glomerular nor extraglomerular immune complex deposits correlated with EC4d levels.
Conclusions: This pilot study revealed that circulating cell and glomerular C4d levels were significantly higher in LN subjects compared to the non-SLE renal controls. In contrast to glomerular and PTC C4d, EC4d levels significantly correlated with LN disease activity. These findings suggest a potential role of C4d on circulating cells as a biomarker for LN.

1506 Immunostaining Findings in IgA Nephropathy: Correlation with Histology and Clinical Outcome in the Oxford Classification Patient Cohort

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Background: IgA nephropathy is defined by the presence of IgA-dominant glomerular deposits. Within this definition there is variation in the location of IgA and the presence of other immunoglobulins. The Oxford Classification of IgA nephropathy identifies 4 histological features that are independent predictors of clinical outcome but does not include immunostains. Here we investigate the potential clinical significance of immunostaining data.

Design: Original biopsy reports from patients in the Oxford Classification study were reviewed. The location of IgA deposits (mesangial vs mesangial + capillary wall) and the presence of IgG >trace were correlated with histological and clinical features.

Results: Original biopsy reports were available for 211 of 265 patients in the Oxford Classification cohort. Of these, 175 included sufficient detail to subclassify immunostaining findings. The presence of capillary wall IgA deposits was associated with a higher mesangial cellularity score (1.28 ±0.65 vs 0.89 ±0.51 for mesangial-only IgA, p=0.004) and more endocapillary proliferation (% of glomeruli 12.2 ±16 vs 5.3 ±12.1, p=0.003). Similarly, the presence of IgG, was associated with a trend to higher mesangial cellularity score (1.16 ±0.64 vs 0.91 ±0.54, p=0.059) and more endocapillary proliferation (10.4 ±14.1 vs 5.1 ±12.1, p=0.005). Capillary wall IgA and the presence of IgG were associated with younger age at diagnosis and greater immunosuppression (IS). The % of patients who received IS was 35% of those with capillary wall IgA vs 23% with mesangial-only IgA, and 37% of those with IgG vs 21% with no IgG. These differences did not reach statistical significance but are a potential source of bias when interpreting the impact of immunostaining findings on clinical outcome. Analysis of follow-up data revealed no significant association between the location of IgA or presence of IgG and rate of loss of renal function, and no association between the location of IgA and renal survival. There was a trend towards poorer renal survival in those patients with glomerular IgG (p=0.055).

Conclusions: We conclude that the location of glomerular IgA and the presence of IgG correlate with mesangial and endocapillary cellularity but do not independently predict clinical outcome. These findings do not support the inclusion of immunostaining data in the Oxford Classification.

1507 Thrombotic Microangiopathy: Clinicopathologic Study of 46 Cases

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Background: Thrombotic microangiopathy (TMA) is a morphological lesion occurring in a group disorders characterized by endothelial injury and thrombi in small vessels and capillaries. Clinically, TTP, scleroderma renal crisis and malignant hypertension are most commonly associated with TMA. Many extrinsic causes such as infection, radiation, VEGF inhibitors and drugs induce TMA. The kidneys are often involved and the renal microvasculature appears particularly susceptible to endothelial injury in TMA. Patients with TMA present with diverse and often ambiguous symptoms. Clinicopathologic correlation is essential for accurate diagnosis since therapies differ. In this retrospective study, the clinicopathologic features in 46 kidney biopsies that revealed TMA were analyzed.

Design: 46 cases of TMA from 3467 kidney biopsies in a 5-year period were identified. LM, IF and EM was performed per departmental protocol. The corresponding clinical records were reviewed.

Results: The results are summarized in Table 1.

Demographic and clinical information	Pathology in renal biopsies	
Age: 46 +/- 14	Acute TMA: 44 (96%)	
Sex: 20/26	Chronic TMA: 2 (4%)	
Acute renal failure: 45 (98%)	Concomitant renal diseases: (20%)	9
Proteinuria: 19 (41%)	AIN	3
Hematuria: 5 (11%)	Diab GN	3
Autoantibodies 5 (11%)	MGN	1
	PIGN	1
	Collapsing GN	1

Forty-five (98%) cases presented with acute renal failure (ARF) or chronic renal disease with rapid decline of renal function. 41% cases had a variable degree of proteinuria. Most cases(>60%)of TTP/HUS and scleroderma had clinical and laboratory results suggestive of TMA. Less than 40% cases in other groups had history or laboratory tests suggestive of TMA. Except for TMA and aging associated vasculointerstitial changes, 20% cases revealed other morphologic lesions that may affect renal function.

Conclusions: TMA is a relatively uncommon finding in kidney biopsies, seen in 1.3 % kidney biopsies. Up to 95% cases clinically presented with ARF. Malignant hypertension, TTP/HUS, scleroderma renal crisis and viral infection were the most common etiologies for TMA. Excluding typical fibrinoid necrosis of glomeruli and/or arterioles, 20% biopsies revealed other pathologic findings in the tubulointerstitial and/or glomerular compartments. Few cases of chronic TMA lack typical light LM morphologic findings of TMA. In these cases, careful EM evaluation and clinicopathologic correlation are needed.

1508 Macrophage in Lupus Nephritis: Can It Be a Predictor of Treatment Response?

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Background: Patients with lupus nephritis class IV who responded to treatment within 6 months had better renal outcome than those who did not. Although parameters such as race, pathologic classification and chronicity index have been associated with treatment response and/or remission, there is currently no information regarding which clinicopathologic parameters can predict treatment response. Glomerular macrophage is known to be associated with clinical parameters indicating poor renal outcome such as higher serum creatinine and proteinuria. The objective of study is to evaluate whether glomerular macrophage number can be used to predict response to treatment in patients with lupus nephritis class IV.

Design: Renal biopsies (N = 92, 88 female) diagnosed with lupus nephritis class IV according to International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 were included in the study. The patients were divided into 2 groups according to response to treatment within 6 months. The treatment response group was defined as having decreased serum creatinine and 24 hr urine protein or UPCR (urine protein creatinine ratio) < 1. The non-response group was defined as stable or increased serum creatinine and/or 24 hr urine protein/or UPCR ≥ 1. There were 46 patients in response group and 46 in non-response group. Immunohistochemistry for macrophage marker (CD68) was performed and the glomerular macrophages were counted on each biopsy. The clinicopathologic parameters were collected including serum creatinine, UPCR and/or 24 hr urine protein at the biopsy time, histologic activity and chronicity indices.

Results: The glomerular macrophage number in response and non-response group was 4.55 ± 2.47 and 6.13 ± 2.51 respectively ($p=0.04$). The glomerular macrophage number was inversely correlated with chronicity index, $r = -0.372$ and $P < 0.001$.

Conclusions: Patients with lupus nephritis class IV who responded to treatment within 6 months had less glomerular macrophages than than those who did not. The glomerular macrophage number may be used to predict treatment response in lupus nephritis class IV patients.

1509 An Animal Model of Kidney Disease Associated with Mitochondrial Dysfunction Made by Collecting Duct-Specific Deletion of CR6-Interacting Factor 1

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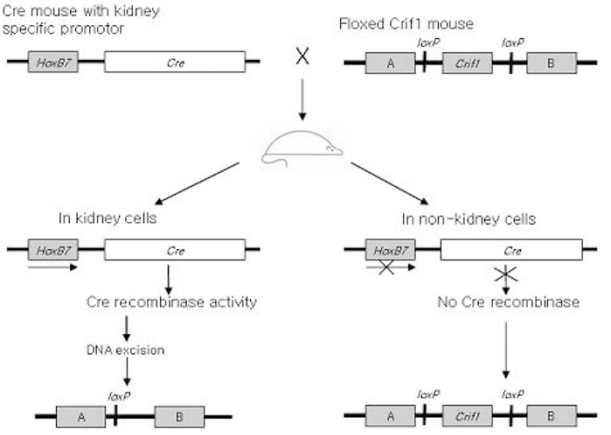
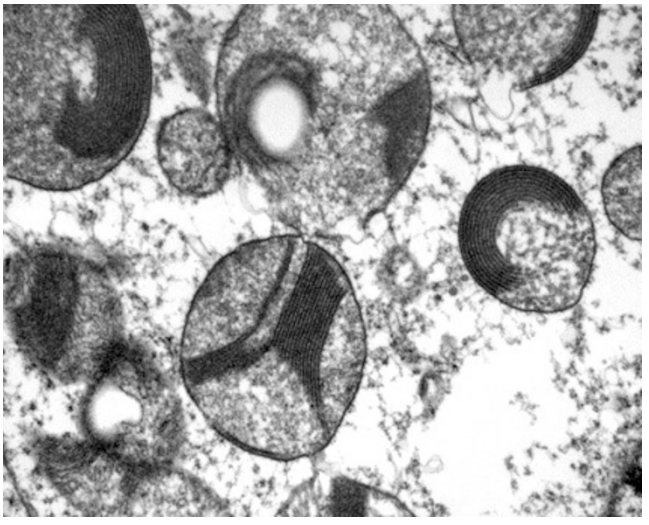
Background: The importance of mitochondria in kidney disease has been recently discovered and an appropriate animal model of mitochondria-associated kidney disease has not been developed yet. CR6-interacting factor 1 (Crif1) is a recently found intranuclear protein which plays an important role in the assembly and stabilization of mitochondria. In this study, we aimed to develop an animal model having mitochondrial dysfunction limited to the kidney by cell-specific gene knock out technique.

Design: To induce collecting duct-specific Crif1 knock out, we used Cre-loxP system. Cre mice having HoxB7 as a collecting duct-specific promotor were mated with Crif1-floxed mice. Among the second generation offsprings, we selected Crif1 homozygously deleted mice and performed the evaluation of physiologic parameters, light microscopic examinations, immunohistochemical stainings for Crif1 and various receptors, and electron microscopic examiniations.



Results: The histologic alteration was not remarkable on light microscopic examinations. However, electron microscopy revealed marked alteration of mitochondria such as size variabilitity, abnormal cristae formation, degeneration and autophagy formation. These features were observed exclusively in collecting duct epithelial cells. The urine volue was decreased and the serum creatinin level was slightly increased.



Conclusions: We developed a collecting duct-specific mitochondrial dysfunction model using Cre-loxP system in mice. This model can be expanded to other mitochondrial disease of the kidney by using another cell-specific promotors (for example, podocin for podocyte).

1510 Validity and Reliability of Renal Cortical Interstitial Fibrosis Assessment by Pathologists on Needle Core Biopsy

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Background: Interstitial fibrosis (IF) is a histological finding that estimates severity of irreversible chronic kidney disease. Pathologists routinely estimate the percentage of IF in kidney biopsies. The accuracy and precision of this determination is unknown. The amount of IF guides clinical decision making between therapy and anticipation of end-stage renal disease. Thus, it is important to know the accuracy and precision of IF assessment of a standard needle core biopsy.

Design: An autopsy kidney from a 66 yo man with chronic kidney disease(eGFR=36) was obtained. Multiple 18-gauge needle core biopsy samples were taken from upper, middle, and lower regions of the kidney. These biopsies (3-4 cores per slide, 3 slides per region) were processed in the usual manner for clinical practice. Three pathologists estimated the percentage (5% intervals) of cortical IF on each slide in a blinded manner. As the gold standard, the entire renal cortex was examined by morphometric methods for IF. Sections of the entire kidney at 0.3 cm intervals were stained for type III collagen. Slides were digitalized using an Aperio scanscope. Quantitation of % IF, with manual delineation of the cortex, was performed by ImageScope pixel count analysis (pixels of type III collagen divided by pixels of cortex). The mean % IF across 27 readings (3 pathologists, 9 slides each) was compared to the % IF by the morphometric method with a t-test. The standard deviation (SD) and coefficient of variation (CV) were used to assess reliability between slides assessed by the same pathologist and between pathologists assessing the same slide.

Results: The mean %IF across the 9 slides was 7%, 12%, and 13% for each of the 3 pathologists, with %IF ranging from 5-25% on each biopsy sample (mean $\pm$ SD 11% $\pm$ 6% overall). This was an underestimate of IF compared to the 14% by the morphometric method (p=.02). The mean SD for multiple pathologists interpreting the same core sample was 4% (CV 41%) and the mean SD for multiple core samples by the same pathologist was 6% (CV 51%).

Conclusions: There is considerable variability in the assessment of IF due to both differences in core samples and differences between pathologists. IF assessment from a single needle core biopsy has reasonable validity but inadequate reliability. These findings have major implications in studies of the progression of fibrosis in renal allografts.

1511 Histopathological Comparison of Idiopathic and Secondary IgA Nephropathy Associated with Liver Cirrhosis

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Background: Immunoglobulin A nephropathy (IgAN), one of the most common causes of primary glomerulonephritis, has been associated with chronic liver disease. So far, the clinical course, optimal management and frequency of IgAN associated with liver disease have not been well established. The aim of this study is to compare the histopathologic characteristics of idiopathic IgAN and that associated with chronic liver disease.

Design: We retrospectively reviewed non-transplant renal biopsy reports and slides of all IgAN cases diagnosed at our institution from 1994 to 2009 (n=57). Eight cases (14%) had IgAN associated with chronic liver disease (group 1). We compared the renal pathology [by conventional staining, immunofluorescence (IF) and electron microscopy (EM)] of group 1 with the rest of the cases with idiopathic IgAN without liver disease (n=49) (group 2). In addition, clinical and demographic parameters available from the patient charts were compared.

Results: Histological examination revealed a significant increase in mempranoproliferative changes (glomerular basement membrane double contour) in group 1. No significant difference was identified between both groups as regards activity and chronicity index and percent of focally or globally sclerotic glomeruli. IF data a revealed significant difference for C1q and no significant difference for C3, IgG, IgA, IgM, Kappa, lambda and fibrin staining. EM revealed a significant increase in reduplication of the glomerular basement membrane, foot processes effacement, subendothelial and mesangial deposits in group 1. No significant difference was noted in subepithelial deposits between the two groups. In terms of available clinical and demographic data, characteristics of the two groups were overall similar. Furthermore, serum creatinine levels at biopsy ranged from 1.2 - 8.9 in group 1. The rate of death from any cause by 6 months post biopsy was 2/8 in group 1. Liver transplantation seems to have lead to better renal function in one out of two patients underwent liver transplantation in group 1.

Conclusions: More mempranoproliferative changes were seen in IgAN associated with liver disease that idiopathic IgAN. This difference is not entirely explained by positive hepatitis C virus serology. These observations may contribute for a better understanding of the natural history of this entity and provide insights for the management of these patients.

1512 Distinctive Renal Biopsy Pathology in the Engraftment Syndrome: A Response to Combined Kidney and Bone Marrow Transplantation

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Background: Renal transplants without maintenance immunosuppression have recently been reported in single-haplotype mismatched living related combined bone marrow (BM)/kidney transplant (tx) recipients, who often develop an idiopathic capillary leak syndrome about 10 days post-tx as donor chimerism becomes detectable, often with spontaneously reversible renal dysfunction. A similar phenomenon seen in both autologous and allogeneic BM txs has been called the "engraftment syndrome (ES)." Here we present novel renal biopsy (bx) findings in this ES.

Design: Indication bxs for graft dysfunction on day 10-12 in an Immune Tolerance Network-sponsored trial (7/9 recipients) were examined by light, immuno- and electron microscopy (EM). Stains included antibodies to Ki67 (MIB-1), CD31, CD68, CD3, and myeloperoxidase. Controls included acute tubular injury (ATI) bxs (n = 5) and normal tx bxs (n = 3) from standard therapy patients at the same time post-tx. Fluorescence in situ hybridization (FISH) was performed in gender mismatched cases for CD45, CD34, and chromosome X and Y.

Results: ES patients had marked ATI, often with interstitial edema, hemorrhage and capillary congestion. Diffuse C4d and focal endothelialitis were each present in 1 case. EM showed glomerular and peritubular capillary (PTC) endothelial injury and loss. CD34 and CD31 stains displayed PTC endothelial cell loss. A marked, significant increase in the number of intracapillary Ki67 cells in glomeruli and PTCs was present compared with ATI bxs. Double staining showed CD31+ and Ki67+ glomerular and PTC endothelial and circulating cells. ATI control cases had mostly tubular cell Ki67 and a similar CD68 cell density. XY FISH showed CD45+ recipient cells in the PTCs with smaller CD34+ proportions.

Conclusions: ES is a spontaneously reversible state with widespread allograft endothelial injury and intracapillary cell proliferation, the latter readily detected with Ki67 and different from ordinary ATI. Whether the mechanism involves a cytokine storm, drug toxicity, and/or allo- or auto-reactivity remains to be defined.

1513 Transplanting Kidneys with Acute Renal Failure and Myoglobinuria; Clinical Correlation and Immuno-Histopathological Findings

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Background: Organ shortage has led to the use of kidneys from donors with impaired renal function. There is limited experience in utilizing kidneys with rhabdomyolysis and little information about the incidence of myoglobinuria in deceased donor kidneys used for transplantation. Histological features of kidney biopsies from myoglobinuric donors and their clinical significance remain largely unclear.

Design: We reviewed 103 immediate post implantation kidney biopsies (within 30 to 60 minutes after revascularization of the allograft) that were obtained since January of 2009. All biopsies (n=15) with features of acute tubular injury (ATI) were reevaluated for the presence of myoglobinuria by immunohistochemistry (IHC). We identified those cases with myoglobinuric ATI (M-ATI; n=5) and classified the remaining cases and by donor source into deceased donor with ATI (DD-ATI; n= 4) and living donor kidneys with ATI (LRD-ATI; n=6).

Results: Myoglobinuric casts in the M-ATI group were coarsely granular and bright eosinophilic; varying degrees of acute tubular cell necrosis were also seen. There were numerous interstitial and some intratubular cells that stained positive for Mib-1 demonstrating high level of proliferative activity. Delay graft function (DGF) was found in 4 of 5 patients from the M-ATI group and 2 of 4 of DD-ATI group. Slow graft function was found in 1 patient from the M-ATI group, 2 from the DD-ATI group as well as 2 patients from the LDR/LTI group. 4 of 6 patients from the LDR/ATI group have immediate graft function. In DD-ATI patients IHC demonstrated the presence of occult myoglobin casts in 4 of the 4 biopsies with minimal findings consistent with ischemic ATN. Living donor kidneys had no demonstrable myoglobin casts. There were no kidney losses in any group of patients.

Conclusions: Rhabdomyolysis appears to be among the acute reversible causes of ATI in donor kidney biopsies and should not contraindicate the use of these kidneys. Myoglobinuria may be more common in transplanted kidneys with ATN than previously recognized. Preliminary data here rendered suggest that more subtle forms of myoglobinuria detected only by myoglobin IHC stain may have a role in early graft dysfunction. Additional research in this topic is warranted to further understand the impact of myoglobinuria in the transplant setting.

1514 Thrombotic Microangiopathy (TMA) in the Setting of Pauci-Immune Crescentic Glomerulonephritis (GN)

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Background: The most common adult crescentic GN is associated with circulating antineutrophil cytoplasmic autoantibodies (ANCAs), which have been directly implicated in this severe renal injury. We have observed TMA in a subset of pauci-immune crescentic GN cases, and we performed a clinicopathologic study to understand the significance of TMA in the setting of pauci-immune crescentic GN.

Design: We reviewed the renal pathology archives over a 3 year period and identified 43 patients with pauci-immune crescentic GN, 10 of which had concurrent findings of TMA. We correlated the histologic findings with relevant clinical information. Only one patient had malignant hypertension, whereas the other nine patients had no known cause of TMA (e.g. thrombotic thrombocytopenic purpura/hemolytic uremic syndrome, scleroderma, or anti-phospholipid antibody syndrome).

Results: Ten of 43 patients (23%) with biopsy-proven, pauci-immune GN had concurrent findings of TMA on histologic examination. All 10 patients presented with acute renal failure, with an average serum creatinine (Cr) of 5.7 mg/dL (range: 2.3-12.0 mg/dL), which did not differ significantly from the 33 patients without evidence of TMA, with an average serum Cr of 4.9 mg/dL (range: 1.2-12.1 mg/dL, p=0.295). Of the 10 patients with pauci-immune GN and concurrent TMA, one presented with malignant hypertension, while 2 others had a long-standing history of hypertension. Other possible etiologic causes of TMA were not identified with the available clinical information. Five patients presented with hematuria, and 2 with nephrotic-range proteinuria. Two biopsies demonstrated focal reduplication of the glomerular basement membranes. Fibrinoid necrosis of arteries and arterioles was present in 4 biopsies. Seven biopsies showed a predominance (>70%) of active lesions, specifically, cellular crescents with necrosis. Four biopsies demonstrated severe acute interstitial inflammation and five demonstrated tubulitis. Interstitial fibrosis and tubular atrophy were patchy and mild in all biopsies.

Conclusions: TMA is often associated with the acute phase of ANCA-associated crescentic GN. These patients are likely to present with markedly increased Cr values, and often with severe hematuria or proteinuria. Recent discoveries of autoantibodies against lysosomal-associated membrane protein-2, a previously recognized ANCA antigen that is abundantly expressed on endothelial cells, suggests that TMA may be an important component to the renal injury that is sustained in pauci-immune crescentic GN.

1515 Proximal Tubulopathy without Crystalline-Like Inclusions: An Important Yet Not Well Recognized Lesion in the Spectrum of Monoclonal Light Chain-Related Renal Diseases

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Background: Light chain-related renal lesions encompass a heterogeneous group of glomerular, interstitial, and vascular manifestations. Proximal tubular damage without crystalline-like inclusions is a poorly recognized member of the group. This lesion is characterized by various degrees of proximal tubular damage associated with a prominent lysosomal system containing enlarged lysosomes, some with atypical shapes. Clinical presentation includes acute renal failure, impairment of normal proximal tubular function, and / or slowly progressive renal insufficiency. Some patients carry a diagnosis of monoclonal gammopathy of unknown significance (MGUS) and are biopsied because of renal dysfunction, in an attempt to determine whether the renal dysfunction is related to the circulating monoclonal light chains.

Design: In an effort to obtain a better idea of the incidence and clinical importance of this lesion, the renal biopsy files of two institutions were carefully examined over a period of three years to identigy cases with this diagnosis. Light, immunofluorescence, and electron microscopy were carefully reviewed in each case.

Results: A total of 1200 renal biopsies were part of the study. lesions associated with nephropathic light chains were collected. Seven cases of proximal tubulopathy were found representing 14% of all monoclonal light chain-associated renal lesions identified. Electron microscopy was crucial in revealing findings suggestive or indicative of the diagnosis. Either immunofluorescence or ultrastructural labeling demonstrated that the lysosomes were packed with monotypical light chains in each case.

Conclusions: Proximal tubulopathy without crystalline-like inclusions is not an uncommon lesion but it remains significantly unrecognized. In patients with a diagnosis of MGUS, this finding indicates that the renal dysfunction is produced by the circulating light chains and that therapeutic intervention is indicated. Because the findings at the light microscopic level may be subtle and immunofluorescence may not be able to demonstrate monoclonal light chains in proximal tubules, ultrastructural evaluation is crucial in some cases. Ultrastructural immunolabeling for light chains to demonstrate monoclonality may be needed to solidify the diagnosis in some cases.

1516 Heavy Chain-Amyloidosis (AH-Amylodosis): Largest Series of a Poorly Recognized Entity

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Background: Less than 10 cases of AH-amyloidosis have been documented in the literature. These have been single case reports with succinct clinical and pathological information. Definition of AH-amylodosis has been restricted to amyloid cases that stain for heavy chains and are negative for kappa and lambda light chains. Whether cases with predominant heavy chain staining but also with monoclonal light chain staining should be included or not in the AH-amyloidosis category or a new category of combined light and heavy chain amyloidosis should be created remains undecided.

Design: Five additional cases of AH-amyloidosis are reported from the files of two institutions during a period of two and a half years in which 1500 renal biopsies were examined, representing the largest series of this entity. A previously reported case by one of the authors is also included for completeness. Clinical and pathological information was collected.

Results: The patients were between 59 and 83 years of age and all of them but one were males. Presentation was proteinuria with or without associated nephrotic syndrome and renal insufficiency. Underlying plasma cell dyscrasias were not recognized prior to the diagnosis of this entity in the renal biopsy. The amyloid material showed light, tinctorial, and ultrastructural characteristics identical to those seen in other types of amylodosis. Four cases were γ and the other two μ-heavy chain related. Two of the cases in addition to predominance of a heavy chain, also had a concurrent monoclonal light chain with equal or less intensity.

Conclusions: AH-amyloidosis is probably more common that recognized. Diagnosis requires careful evaluation of the immunoglobulin fluorescence stains. Clinical presentation did not differ from cases of AL(light chain-related) amyloidosis. None of the patients had a monoclonal gammopathy diagnosed prior to the renal biopsy. All patients are males. Although the definition of AH-amyloidosis up to now has required negative stains for kappa and lambda light chains, it could be broadened to include all cases of amyloidosis with predominant heavy chain staining, or a new categoy of light/heavy chain (ALH) amyloidosis should be created; the latter is more appropriate at this time and we propose to create this new category. The significance of the concurrent monoclonal light chain detection in the amyloid deposits in 2 of the cases is not clear at this time. The pathogenesis of renal AH-amyloidosis in unclear, but is probably similar to that of AL-amyloidosis.

1517 Quantitatitive Spectrum of Glomerular Capillary Double Contours in Transplant Glomerulopathy

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Background: In the Banff 07 classification of Renal Allograft Pathology transplant glomerulopathy (TxGP) scores (cg) are based upon the extent of glomerular capillary double contours (CDC) in the most severely affected of non-sclerotic glomeruli. The cg scores are 0, 1, 2, and 3, corresponding to fewer than 10%, up to 25%, up to 50%, and more than 50% capillary loop involvement, respectively. However, data regarding the spectrum of glomerular CDC in TxGP are sparse, which hampers the development of alternative scoring systems that might prove to be of clinical utility.

Design: The aim of the study was to quantitatively assess the morphologic spectrum of glomerular CDC in 20 consecutive biopsies featuring TxGP in patients with first time TxGP diagnosis. All glomeruli in the paraffin material were scored based on the percentage of capillary loops showing CDC ("CDC score") on the PAS-stained sections. Mean values of CDC with standard deviation were calculated for each case and the mean CDC for all cases was also determined. The findings were correlated with the Banff 07 cg scores of TxGP, and with relevant clinical and morphological variables. The inter-observer reproducibility of the CDC scoring was also evaluated.

Results: Seventeen glomeruli were evaluated on average in each case. The mean CDC score for the 20 cases was 52% (range: 2-97) with a mean standard deviation (SD) of 21% (range 4-36). In 13 of 20 cases the Banff 07 cg scores correlated with the mean CDC score (i.e., the mean CDC score for individual cases was within the percent range as defined by Banff 07 for specific cg scores). However, in 7 out of 20 biopsies (35%) the mean CDC scores were below the diagnostic range of the cg score for the case. There was no correlation of CDC scores with C4d status, post-transplant time, or serum creatinine levels. Inter-observer concordance was excellent for the CDC scores.

Conclusions: These preliminary data suggest that mean CDC scores in TxGP may be considered for testing as an alternative to cg scores of Banff 07. Furthermore, additional exploration of other clinical and morphological parameters should begin to address clinical utility.

1518 Renal Allograft Plasma Cells, B Cells, Banff Grade and Outcome

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Background: Recently, interest has been generated in the presence of plasma cells and B cells in renal allograft rejection, but their role remains unclear. In particular, this includes the intragraft relationship between these cells, and whether these cells contribute to acute cellular rejection (ACR), acute humoral rejection (AHR), or both. Some association with poor outcome has been shown. Our aim was to examine the association, if any, between plasma cells and B cells, their relationship with the type of rejection, and whether an assessment of their presence is useful as a determinant of outcome.

Design: We identified all cases of acute rejection between 2005 and 2009 accompanied by noticeable (≥ 5%) plasma cell infiltrates (n=23), and selected 10 cases of varying grades of acute cellular rejection as controls. Histopathology, C4d by immunofluorescence, B and T cells by immunohistochemistry (CD20 and CD3 respectively), and graft outcome were evaluated.

Results: Significant plasma cell infiltrates as part of acute rejection were seen in biopsies performed from 24 days to 7.5 years (median, 20 months) after transplant; 7 cases had 5-15% plasma cells, while the remaining 16 cases had ≥25% plasma cells (plasma cell rich [PCR]). Seventeen cases were Banff-Grade 1, and 6 cases were Grade 2. Ten cases had concomitant AHR. T cells accounted for most of the lymphocytic infiltrate in all cases. B cells ranged from 1 to 30% both in cases with and without plasma cells. Higher percentages of B cells were seen when aggregates of lymphocytes were present, whether of a follicular nature or not. Extranodular B cells varied from 1 to 7%, and were always ≤2% of the total infiltrate when aggregates were absent. Eleven grafts were lost at a mean of 3.5 years after transplant. C4d positivity, PCR-ACR and increased B cells were associated with poor outcome, although independent association was seen only with C4d and PCR-ACR.

Conclusions: Plasma cell rich rejection has a poor outcome independent of the Banff grade of rejection. The frequent presence of concomitant AHR may suggest

a pathogenetic link. Increased B cells are suggested by the presence of lymphocytic aggregates on H&E stain, although the understanding of intragraft B cells remains unclear.

1519 Polyoma Virus Nephropathy and Acute Rejection in Renal Allografts

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Background: Polyoma virus nephropathy (PVN) of the renal allograft is associated with tubulointerstitial inflammation that may represent viral interstitial nephritis. The distinction of graft inflammation in PVN from acute rejection (AR) may be difficult. Intimal arteritis and peritubular capillary C4d expression are hallmarks of rejection. This study describes features of simultaneous PVN and AR in renal allograft biopsies.

Design: All allograft biopsies with PVN (n=144 from 94 patients) received between June 2001 and March 2009 were included. PVN was staged using Drachenberg criteria. Ten had features of AR, by Banff criteria, in addition to PVN. Clinical data were obtained by chart review.

Results: Group 1 (n=4) had AR identified after immunosuppression was lowered for the treatment of prior PVN. Three had type 1B AR with PVN stage A (n=1) and B1 (n=2). One had diffuse peritubular capillary (PTC) C4d with PVN stage C. Group 2 (n=6) had AR and PVN arising without change of immunosuppression (type 1 n=2, PVN stages A and B1; type 2A n=3, PVN stages A, B1, B3; and type 2A with diffuse PTC C4d n=1, PVN stage B2). Graft loss was 25% in group 1 with 1-33 months follow up, and 60% in group 2 with 5-60 months follow up (p=0.5). Graft loss in biopsies with type 1 AR and PVN was 40%, and with type 2A AR ± PTC C4d and PVN was 50% (p=1).

Conclusions: AR can be observed with PVN as a result of lowered immunosuppression for treatment of PVN or these lesions may arise spontaneously. Criteria for rejection in this setting include intimal arteritis and peritubular capillary C4d staining. Disproportionately severe tubulointerstitial inflammation with focal PV infection may be a potential criterion for AR.

1520 ALH Amyloidosis: An Underrecognized Diagnostic Entity

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Background: In the renal biopsy workup of AL amyloidosis, variable IgG immunofluorescence (IF) staining of the amyloid deposits can be seen. We conducted the following study to determine the significance of this observation.

Design: Our pathology archives were reviewed from 2005 to 2008 and renal biopsies of AL (n=24) and AH (n=1) amyloidosis were identified of which were 11 biopsies had at least 1+ staining for IgG (DAKO). Direct IF microscopy using FITC-conjugated antibodies to IgG1, IgG2, IgG3, and IgG4 (Binding Site, San Diego, CA) was performed. The slides were scored semi-quantitatively from 0-4+.

Results:

IF staining results

Case	IgG	Light chain	IgG1	IgG2	IgG3	IgG4	SPEP
1	2-3+	lambda 3-4+	0	0	0	0	IgG lambda
2	3+	lambda 3+	0	0	2-3+	0	N/A
3	3+	lambda 3+	0	0	0	0	IgG lambda
4	trace-1+	kappa 1-2+	0	0	0	0	IgG kappa
5	1-2+	lambda 3+	0	0	0	0	lambda only
6	1-2+	lambda 3+	0	0	0	0	N/A
7	trace-1+	kappa 2+	0	0	0	0	IgG kappa
8	1+	lambda 3+	0	0	0	0	N/A
9	1-2+	kappa 3+	0	0	0	0	N/A
10	3+	0	0	1-2+	0	0	Negative

Abbreviations: SPEP - serum protein electrophoresis; N/A - not available

Electron microscopy confirmed the presence of amyloid fibrils in all cases and did not show evidence of monoclonal immunoglobulin deposition disease. When the intensity of IgG IF staining of amyloid deposits was stronger than albumin but less than the corresponding monoclonal light chain, the absence of IgG isotype staining was interpreted as a non-specific finding. Two patients had circulating IgG lambda paraproteins with strong (3+) IF staining for both IgG and lambda light chains but no staining for IgG1, IgG2, IgG3, or IgG4. Overall, 20 biopsies (80%) of AL (15 lambda, 5 kappa light chain), 4 (16%) ALH, and 1 (4%) AH amyloidosis cases were identified.

Conclusions: Similar to light and heavy chain deposition disease, ALH amyloidosis is an appropriate term for amyloid deposits consisting of both a monoclonal heavy and light chain component. This diagnostic subtype of amyloidosis may be underrecognized. Additional antibodies to IgG1, IgG2, IgG3, and IgG4 should be studied to determine if discrepant IF staining results between pan-IgG and the IgG isotypes in select amyloidosis cases could be due to significant mutation of the heavy chain component, conformational change of the amyloidogenic heavy chain, stearic hindrance, or trapping of non-amyloidogenic heavy chains.

1521 Prevalence and Morphology of Leukocyte Chemotactic Factor 2-Associated Amyloid (ALECT2) in Renal Biopsies

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Background: Nephropathologists typically identify the protein nature of renal amyloid deposits immunohistochemically utilizing antisera reactive to amyloidogenic precursors. The majority of amyloid cases can be typed with a simple antibody panel including immunoglobulin light chain (κ or λ) and serum amyloid A. However, in some instances, these reagents do not recognize the congophilic material or yield ambiguous staining results, thus creating a diagnostic dilemma. Chemical analysis of fibrils extracted from just such a 'non-reactive' renal biopsy sample led to the discovery of a previously unknown type of amyloid formed from leukocyte chemotactic factor 2.

Design: Among the 21,598 kidney biopsy specimens sent to our laboratory over the past 8½ years, 285 (1.3%) were found histochemically to contain apple green birefringent congophilic deposits that, by electron microscopy, had the typical ultrastructural features of amyloid. Thirty one of these amyloid cases could not be typed immunohistochemically. In an effort to determine if any of these 31 unclassified amyloid-containing samples were LECT2-related, tandem mass spectrometry was performed.

Results: Seven of the 31 cases were identified as ALECT2 (overall 2.5%); this finding was confirmed immunohistochemically using an antiserum specific for LECT2. Pathologically, the deposits were strongly congophilic and had distinctive morphologic features. These features include extensive interstitial involvement in each and, with 1 exception, marked diffuse, global mesangial expansion and displacement by the congophilic deposits.

Conclusions: The extensive interstitial and glomerular involvement, as well as the failure of the amyloid to be immunostained by conventionally used antisera, may indicate the presence of ALECT2. ALECT2 represents the third most common form of renal amyloidosis in our series.

1522 Focal Segmental Glomerulosclerosis and Extra Efferent Vessel Development in IgA Nephropathy

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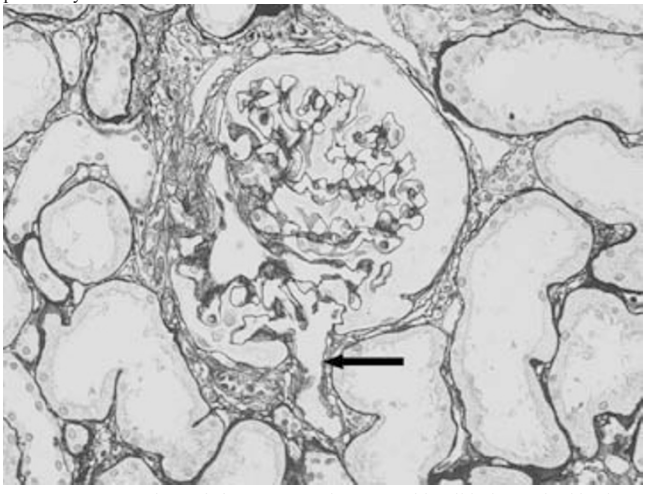
Background: Segmental glomerulosclerosis (SGS) is not infrequently observed in advanced stage of IgA nephropathy (IgAN) with severe mesangial/endocapillary proliferation or interstitial fibrosis. However, the morphology and pathogenetic mechanisms have not been clearly demonstrated in cases showing mild glomerular and tubulointerstitial changes.

Design: To explore the evolution of SGS in this condition, we selected 62 cases of IgAN with focal SGS, which did not demonstrate significant mesangial or endocapillary proliferation or tubulointerstitial fibrosis (Hass subclass II). Each segmentally sclerotic glomerulus was traced in more than 20 serial sections and SGS was subtyped as classic, perihilar, tip, cellular, and collapsing variant. These data were correlated with other histologic and clinical parameters.

Results: SGS was present 2.6% to 45.5% of glomeruli in each case. Among the five subtypes, tip variant was the most common (31 cases) followed by classic type (29 cases) and perihilar variant (12 cases). Neither cellular nor collapsing forms were present. Adhesion of capillary loops was present in almost all cases. Most of the tip lesions appear to be similar to glomerular scar associated with sclerosing capillaritis. Interestingly, we observed extra-efferent vessels situated away from vascular hilum, which communicated glomerular capillaries and extraglomerular capillaries through gaps in Bowman's capsule, a phenomenon which has not been reported in literature previously.



Conclusions: SGS is not infrequent even in IgAN with mild glomerular histology. Most of the SGS seem to result from capillary endothelial injury. However, it should be further clarified on the mechanisms of formation of extra-vessels through gaps in Bowman's capsule in IgAN.

1523 Thin Glomerular Basement Membrane: Histopathologic, Ultrastructural, and Molecular Variability

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Background: Thinning of glomerular basement membrane (GBM) is encountered in about 1% of medical renal biopsies. The most frequent and important question is whether the findings represent Alport. Collagen IVα3 and α5 immunofluorescence (IF) and electron microscopy (EM) can be very helpful in many cases. Alport variants not infrequently are a diagnostic challenge because of variable IF and EM findings. We present the collagen IVα3,5 and EM results of thirty renal biopsies with segmental or diffuse GBM thinning.

Design: Light microscopy, routine and specific IF for Collagen IVα3, α5 minor chains, and EM of 30 renal biopsies from our consult files were reviewed. Demographic data (age, sex, family history and presenting symptoms) were tabulated along with detailed histopathologic findings. GBM thinning was determined based on age and previously determined threshold of ≤ 200 nm.

Results: All patients presented with gross hematuria. Twenty patients (66%) also had varying degrees of proteinuria; three of nephrotic range. Fourteen patients were male and 16 female. The age ranged from 4 to 51 years old (mean = 19.7). Only 5 patients had definitive family history of renal disease (16.4%). Under light microscopy examination, 14 patients showed unremarkable glomeruli (47%), 8 patients showed only mesangial hypercellularity (27%), 5 showed increased global glomerulosclerosis (17%), and 3 had

FSGS (10%). IF for α3, α5 (IV) results were as follows: +/+ in 15 biopsies (50%), -/- in 3 (10%), -/+ in 2 (6%), +/- in 1(3%), both chains mosaic in 4 (13%), both decreased in 1. The remaining 4 biopsies had other staining combinations. EM showed thin GBM in all cases; 16 patients showed lamellation within thin segments, 12 of these also had bread crumbs; 14 had diffuse thinning with no lamellation;18 biopsies showed varying degrees of foot process effacement. The diagnosis of X-linked Alport was made in 6, Autosomal-recessive Alport was suggested in 13 (total Alport 19/30=63.5%), TMD in 11/30 (36.6%), of which superimposed glomerular disease was present in 6 (45%): minimal change disease in 3, FSGS in 2, and post-infectious in 1.

Conclusions: In this cohort the majority of thin GBM are either X-L Alport or Alport variant. A significant 45% of TMD was concurrent with another primary glomerular disease which appears to be the likely cause of presenting symptoms. Therefore, TMD in this population was a diagnosis of exclusion. Alternatively, GBM thinning may underline the pathogenesis of glomerular diseases such as FSGS and minimal change disease.

1524 The Reduction or Elimination of Tissue Autofluorescence in Formalin-Fixed, Paraffin-Embedded Renal Biopsy Images Using Spectral Imaging

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Background: For half a century, immunofluorescence (IF) on frozen sections has been the gold standard for immunochemical evaluation of renal biopsy specimens. Immunofluorescence (IF) labelling is not often used for formalin-fixed, paraffin-embedded (FFPE) specimens, the perception being that the inherent autofluorescence of such specimens makes high quality IF imaging difficult. This has placed two severe restrictions on investigators. First, it has limited fluorescence imaging to tissue cryosections and hence restricts analysis of clinical material. Second, as cell and tissue preservation is lower in cryosections than in FFPE sections, the quality of morphological findings is frequently compromised. However, advances in spectral imaging algorithms can provide a simple means of overcoming one of the major limiting factors on sensitivity and legibility of IF imaging of FFPE specimens, viz., tissue autofluorescence.

Design: A comparison between IF images taken of standard frozen-section renal biopsies and IF images taken of FFPE renal biopsy sections using a spectral imaging system was made for a variety of immunoglobulins and complements. Unlabeled FFPE sections and were used to develop spectral signatures of tissue autofluorescence, which were then used in conjunction with positive IF sections to develop the spectral signatures of the immunostain used (FITC).

Results: By utilizing correct spectral signatures of tissue autofluorescence and the immunostain (FITC), spectral imaging was shown to improve the contrast ratio in IF images of FFPE specimens by up to 100-fold, greatly increasing legibility and sensitivity. In addition, IF of FFPE sections had improved morphology compared to frozen sections.

Conclusions: The use of spectral imaging for acquiring IF images is an easy-to-implement methodology on any standard fluorescence microscope, and requires little to no extra time for imaging compared to regular imaging methods. By eliminating or greatly reducing the impact of tissue autofluorescence (by as much as two orders of magnitude) on image quality, spectral imaging can enable a shift from utilizing frozen sections for IF of renal biopsy specimens to utilizing more easily obtainable and preferable FFPE specimens.

1525 Revisiting the Banff Scores for Renal Allograft Pathology

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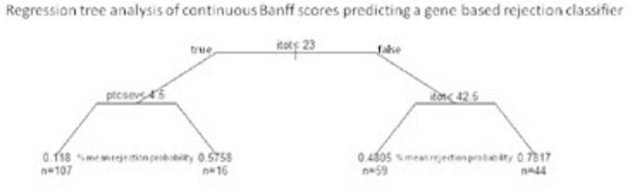
Background: The Banff classification represents the international diagnostic consensus in renal allograft pathology. In the different morphological compartments of the kidney lesions are quantified and assigned a score of 0-3 according to arbitrary thresholds. Lesions scores are then again arbitrarily assembled into diagnosis with direct clinical implications. Consensus rules and thresholds for scoring lesions and assigning diagnosis were developed empirically in 1991 and later clinically validated by correlating them with response to treatment and outcome. However, significant advances in treatment and clinical management occurred since then. Thus we aimed to revisit the Banff scoring criteria and thresholds in terms of their current diagnostic and clinical utility.

Design: We assessed in 226 renal allograft biopsies for cause the extent and severity of lesions in interstitium, tubuli, vessels, capillaries and glomeruli as continuous variables. Using Principal Component and Regression Tree Analysis we compared Banff scores and continuous scores with each other and in terms of their predictive value for a molecular rejection classifier and allograft survival.

Results: Banff scores and continuous variables grouped closely together in Principal component analysis. The total i-score, i.e. the extent of all cortical inflammation was best predicting a high probability of rejection based on a recently described molecular rejection classifier. Interestingly the regression tree analysis suggested a total i-score threshold as the most predictive (>23%) similar to the current Banff cut-off for rejection (figure1). In terms of allograft survival the extent of tubular atrophy turned out to be the best predictor, at a threshold (>25%) identical to the current Banff ct2 score.



Conclusions: Continuous re-assessment of renal allograft lesions revealed total cortical inflammation as the best predictor of the molecular burden of inflammation and injury. The extent of tubular atrophy was a robust predictor of allograft survival. Surprisingly for both lesions the unsupervised regression analysis suggested thresholds virtually identical to the empirically derived Banff scores and thus independently confirms their clinical value.

1526 Noninvasive In Vivo Assessment of Renal Fibrosis Using Collagen I Luciferase Reporter Mice

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Background: Nep25 transgenic mice express the human CD25 receptor on podocytes, and develop progressive glomerulosclerosis when immunotoxin (LMB2) is administered and binds this receptor. By cross-breeding to mice with luciferase inserted in the collagen type I promoter, we aimed to create a model in which expression of collagen I can be monitored by non-invasive imaging.

Design: Collagen I-luciferase/Nep 25 mice were exposed to a dose range of LMB2 toxin (10, 15 or 20 ng/g BW, i.p.). Mice were biopsied at week 2 and sacrificed at week 6. Body weight, urine albumin/creatinine ratio (ACR), serum creatinine, glomerulosclerosis index (SI, 0-4 scale) and collagen I staining were measured. Mice were imaged after luciferin i.p. injection at intervals for detection of luciferase activity, expressed as intensity at 560nm wave length.

Results: Low dose LMB2 temporarily induced proteinuria (ACR, baseline 20.4±6.9, week 1 582.4±340.2), which spontaneously resolved (week 6 43.4±15.8), with no glomerulosclerosis. Medium dose LMB2 induced edema and severe proteinuria (ACR, week 1 966.4±528.2, week 6 981.6±38.6). Moderate glomerulosclerosis was found at biopsy at 2 weeks (SI 2.01±0.17), and progressed at 6 weeks (SI 2.50±0.21). High dose LMB2 induced not only edema and proteinuria (week 1 677.2±132.6, week 6 2356.6±494.9), but also severe glomerulosclerosis (SI, biopsy 2 weeks 2.54±0.20, autopsy 6 weeks 2.92±0.33), high serum creatinine (baseline 0.24±0.05, week 6 0.56±0.09 mg/dl, $P<0.05$) and BUN (baseline 16.69±20.41, week 6 262.81±40.24 mg/dl, $P<0.05$). The luciferase imaging score increased over time (baseline 6.23±0.80, week 2 16.37±2.20, week 3 16.77±4.15, week 4 18.77±2.00, week 5 22.54±2.02, week 6 31.24±5.55 Xe^7). Glomeruli and interstitium showed increased collagen I immunostaining over time, which correlated with luciferase imaging score in vivo (R^2=0.94).

Conclusions: Collagen I-luciferase/Nep 25 mice provide a non-invasive model for monitoring renal fibrosis, with dose-dependent proteinuria and glomerulosclerosis as well as increased collagen I imaging signals. This model will allow dynamic longitudinal monitoring of renal scarring and response to interventions in vivo.

1527 Immunophenotyping Interstitial Inflammatory Cells in Acute Rejection

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Background: The potential role of B lymphocytes and plasma cells (PCs) in antibody-mediated (humoral) rejection is gaining attention, but remains poorly understood. We hypothesize that significant accumulation of intraparenchymal B and PCs may occur in acute antibody-mediated rejection and attempt to characterize the infiltrate in a different manner than those in the literature.

Design: We searched our pathology archives from 2005-2009 and identified 57 human renal biopsies with a diagnosis of acute rejection. The biopsies were divided into 3 groups: 1) cell-mediated rejection only; 2) antibody-mediated rejection only (with positive C4d peritubular capillary deposition); and 3) both cell- and antibody-mediated rejection. Standard immunohistochemistry was performed on paraffin tissue sections using monoclonal antibodies to CD20, CD138, CD3 and CD45 (DAKO, Carpinteria, CA). The number of interstitial cells with strong membranous staining was counted for each antibody and the percentage of positive cells was calculated using the total positive CD45 cells as the denominator. This data was correlated with clinical information, biopsy diagnosis, and presence of HLA antibodies. Statistical analysis was performed using the Mann-Whitney or Kruskal-Wallis test with $p<0.05$ considered as significant.

Results:

Immunophenotype of interstitial inflammatory cells

Rejection type	CD20%	CD138%	CD3%	# of cases
Cell-mediated	13.6%	5.1%	75.6%	21
Antibody-mediated	13.0%	6.9%	57.4%	15
Cell- and antibody-mediated	15.7%	15.8%	57.9%	21
C4d positive	14.7%	11.9%	57.2%	30
C4d negative	12.3%	5.2%	75.8%	27
HLA class I/II abs present	11.1%	10.8%	66.8%	27
No HLA class I/II abs	25.9%	1.5%	78.6%	4

The average percentage of PCs in biopsies with or without C4d peritubular capillary deposition was 11.9% and 5.2%, respectively ($p=0.01$). The combined cell- and antibody-mediated rejection biopsies demonstrated more than twice as many PCs than the other two groups ($p=0.008$). The average percentage of PCs in patients with the presence or absence of anti-HLA class I or II antibodies was 10.8% and 1.5%, respectively ($p=0.03$).

Conclusions: Significantly increased percentages of interstitial PCs were observed in renal biopsies with both cell- and antibody-mediated rejection, presence of C4d peritubular capillary deposition, and presence of HLA class I or II antibodies. Intrarenal PCs deserve further attention to understand their potential role in antibody-mediated rejection.

1528 Comparison of Tacrolimus and Cyclosporine Nephrotoxicity in Native Kidneys of Diabetic Pancreas Transplant Recipients

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Background: Nephrotoxicity is a major adverse effect of tacrolimus (TAC) and cyclosporine (CSA), two important calcineurin inhibitors (CNI). Severe CSA-associated renal lesions develops within 5 years in pancreas transplanted (PT) patients. However, there is little information on TAC-induced lesions in native kidneys. We evaluated renal structure and function in formerly type 1 diabetic subjects with successful PT treated with TAC or CSA.

Design: Protocol kidney biopsies before and 5 years after PT in 14 patients receiving TAC and 12 patient receiving CSA were compared for interstitial volume fraction [Vv(Int/cortex)], % sclerosed glomeruli (SG), and index of arteriolar hyalinosis (IAH) on PAS stained slides. Glomerular filtration rate (GFR), blood pressure, TAC and CSA dose and blood levels were repeatedly monitored. Patients were on CSA or TAC for at least 4 years.

Results: TAC and CSA groups were comparable for all baseline clinical features and LM parameters except for age, being higher in TAC. GFR decline from baseline to 5 years was not statistically different for TAC (44%) and CSA (33%). Vv(Int/cortex) increased 24% ($p<0.001$) at 5 years post-PT in TAC and 26% ($p<0.005$) at 5 years in CSA patients. %SG increased from 6.1±7.5 % at baseline to 30±17.2 % at 5 years ($p<0.0001$) in TAC, and from 9.1±10.6% to 35.6±19.3 %, in CSA group ($p<0.002$). IAH did not significantly change in either the TAC or CSA group from baseline to 5 years. Baseline to 5 year changes of Vv(Int/cortex), %SG and IAH were not statistically different between TAC and CSA patients. There were significant inverse correlation between the decrease in GFR and the increase in Vv(Int/cortex) ($r=0.68$, $p=0.005$) in TAC group. There was no correlation between any of the TAC dose and blood levels and changes in renal structural and functional parameters, but CSA dose and blood levels in the first post-PT year were significantly correlated with GFR decline and Vv(Int/cortex) increase from baseline to 5 year follow-up.

Conclusions: This study demonstrates that in type 1 diabetic native kidney successful pancreas transplant recipients, the chronic nephrotoxic effects of TAC are similar to those of CSA both in terms of renal function and of renal structure. Alternative immunosuppression strategies could improve the risk to benefit ratio of PTA.

1529 Crystalline Nephropathy Due to 2, 8-Dihydroxyadeninuria: An Under-Recognized Cause of Irreversible Renal Failure

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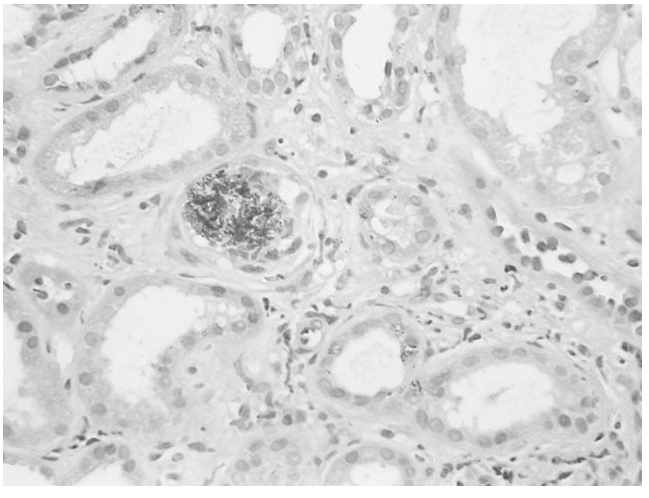
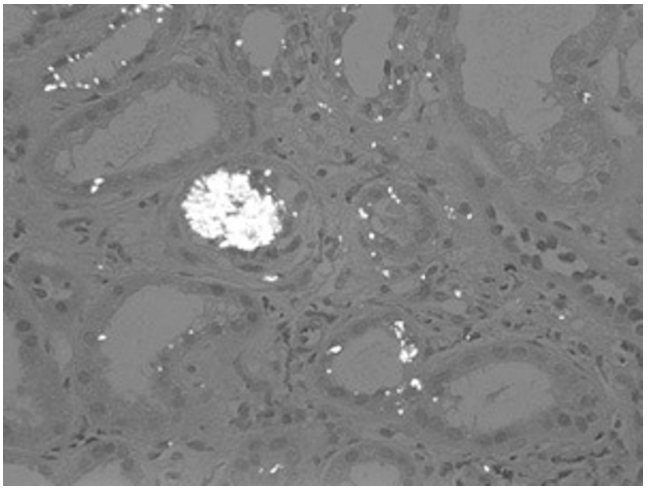
Background: 2, 8 dihydroxyadeninuria (DHA) is a rare autosomal recessive disorder caused by APRT deficiency, typically manifesting as recurrent stones. Only rare cases of DHA stones have been reported from the U.S. Here, we report 3 American pts with DHA crystalline nephropathy leading to ESRD with recurrence in the allograft.

Design: 3 cases of DHA crystalline nephropathy were identified from our Nephropathology archives.

Results: All 3 pts were white adults with no history of obstruction. 2 pts had no history of stones and 1 had a single episode of stones 36 yrs prior to presentation. All pts presented with renal failure with a mean creatinine of 7.5 mg/dl. Renal biopsies showed numerous tubulointerstitial DHA crystals, tubular degenerative changes, and moderate tubulointerstitial scarring. DHA crystals stained brown with H&E [fig 1] and were strongly birefringent [fig 2]. In the tubular lumina, they formed fan-like or irregular shapes. Within the tubular cytoplasm, they appeared as small single crystals with rod or rhomboidal shapes. In the interstitium, both single crystals and aggregates were seen. 2 pts were initially misdiagnosed, one as primary hyperoxaluria and the other as chronic interstitial nephritis. The diagnosis of DHA disease was confirmed by finding complete absence of APRT enzyme activity on blood spot. All 3 pts progressed to ESRD, within one month following biopsy in 2 and after 9 months in 1. All 3 pts underwent renal transplant with early disease recurrence in 3 allografts in 2 pts.

Conclusions: DHA disease is an under-recognized condition that can lead to irreversible renal failure and recurs in the allograft. It must be considered in the differential diagnosis of crystalline nephropathy, even in the absence of history of stones. Pathologists should be familiar with the characteristic brownish and birefringent tubulointerstitial crystals of this rare condition.





1530 Maternal Undernutrition Dysregulates Apoptosis in Offspring Fetal Kidneys

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Background: Maternal undernutrition (MUN) results in offspring kidney nephron deficits but the mechanisms of this phenotypic change are unknown. We sought to evaluate alterations in MUN-induced fetal kidney RNA and protein expression to assess dysregulation of apoptosis in this model.

Design: Pregnant rat dams were 50% food restricted from embryonic day (E)10. Male offspring E20 and postpartum day (P) 1 kidneys were removed. E20 whole kidney RNA was hybridized using rat Agilent DNA microarrays. E20 and P1 kidneys were frozen for Western blot analysis. P1 kidneys were fixed in paraformaldehyde for TUNEL staining and assessed semiquantitatively (0-4+ scale).

Results: E20 kidney microarray assessment showed 198 known genes with ≥ 1.5 fold change, which were analyzed for placement into ontological groups and signaling pathways. Four significantly upregulated genes were detected in apoptotic signaling pathways; Fas ligand (7.0 fold), TNFα1 (3.5 fold), PI kinase (6.2 fold), and AKT2 (1.5 fold). To further assess apoptosis, Western blots were performed for these proteins as well as down-stream effector genes. E20 kidneys had upregulation of Fas (2.2 fold, $p < 0.002$) and Bcl-2 (1.8 fold, $p < 0.003$) relative to controls (C). P1 kidneys demonstrated augmented Fas (1.9 fold, $p < 0.05$), BAX (4.5 fold, $p < 0.01$) and caspase 3 (1.6 fold, $p < 0.03$) with significant decrease in Bcl-2 (0.6 fold, $p < 0.01$). Fas ligand protein was not significantly altered at E20 (1.4 fold) or P1 (1.3 fold). TUNEL stain in P1 kidneys revealed apoptosis predominantly in the nephrogenic zone and the deep medulla/papillae. MUN P1 kidneys had significant increase in nephrogenic zone TUNEL staining (MUN 2.2 ± .3 vs C 1.6 ± .5, $p < 0.05$); deep medullary TUNEL was not significantly different.

Conclusions: This study demonstrates upregulation of apoptosis in MUN offspring kidneys prominently in the nephrogenic zone, with differences in apoptosis signaling before and after birth. Pro-apoptotic proteins were augmented, while anti-apoptotic Bcl-2 was increased at E20 but reduced at P1 suggesting an effect of parturition on cell survival. Previously, we have demonstrated downregulation of Notch in MUN E20 kidneys; Notch determines cell fate and impacts apoptosis. Therefore, in the late fetal period altered apoptosis may be, in part, a downstream effect of Notch dysregulation and/or a direct effect of MUN. Increased apoptosis likely is an important mechanism inducing nephropenia in MUN progeny.

1531 Lupus Nephritis in Iran

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Background: Renal involvement is one of the most serious complications of systemic lupus erythematousus (SLE) and the prevalence of Lupus nephritis is reported to be 40-75% of patients in different studies. Moreover, there have been differences in the severity of clinical and the prevalence of pathological findings in different geographical regions. However, our data concerning Iranian patients are rare.

Design: In this study, we reviewed known cases of SLE, according to American college of Rheumatology, who were admitted to Hasheminejad Kidney Center from 1998 to 2007 and underwent a kidney biopsy. After verifying the diagnoses based on clinicopathological findings, 153 cases met the entry criteria. All the patients' biopsies were reviewed by a single nephropathologist and were classified according to WHO classification. Also, clinical and laboratorial findings of the patients were evaluated.

Results: A total of 153 patients (125 female, 28 male) were the subjects of this study having an average age of 29.3±11.4 (range 12-71 years).The most common clinical manifestations were arthritis, hypertension and malar rash (80, 52.3, 45.2% respectively).The prevalence of major renal syndromes including nephrotic and nephritic syndromes were 55% and 23.5 %, respectively. The mean level of serum creatinin was 2.26 mg/dl and 48.4% had high serum creatinin (1.5 mg/dl). Anemia (Hb<12gr/dl), Thrombocytopenia (Plt. <100000/l) and leukopenia(WBC<4000/l) were detected in 80%, 13.3% and 22.2% of patients, respectively. Low serum C3, C4 and CH50 were found in 63.4, 54.9 and 55.6 %, respectively. Patients with Class IV lupus Nephritis had the most frequency of hypertension (53%), low C3 (56%) and a high level of creatinin (54%). Antinuclear antibody was found to be 81% and anti-ds DNA antibody was

detected in 53.1% of cases. 5.7 % of patients were ANCA positive. Histopathologic classification of the patients according to WHO criteria were as follows: class II 9.9%, class III 27%, class IV 55.3%, class V 7.8%.

Conclusions: Our study supports the correlation between some of the clinical and laboratorial findings of the patients with the histopathologic classification. The most common histopathologic class of our study was class IV (diffuse lupus nephritis) that was similar to other reports from France, USA, Netherlands and Thailand. We also compared our findings with other studies in Middle East and to our surprise we found that, unlike our study, the most frequent lupus nephritis class in studies from United Arab Emirates, Saudi Arabia and Kuwait was Class III.

1532 Glomerular, Tubular, and Urinary Immune Complexes (ICs) in IgA Nephropathy (IgAN): High-Resolution Image Analysis and ELISA Studies

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Background: IgAN patients have glomerular IgA1-containing deposits originating from circulating ICs composed of aberrantly glycosylated IgA1 bound by anti-glycan antibodies (IgG or IgA1). We hypothesized that these circulating ICs may escape hepatic clearance, deposit in the glomeruli, and induce renal injury. Furthermore, a fraction of these ICs may be excreted in the urine. The properties of these renal immune deposits have not been well studied in terms of the pattern, co-localization of the components, and presence in tubuli.

Design: Frozen renal biopsy specimens from 10 randomly selected IgAN patients were stained with fluorochrome-labeled antibodies against IgG, IgA, and C3. For seven patients, urine was obtained contemporaneously, *i.e.,* just before renal biopsy. Urinary IgA-IgG complexes were measured by ELISA. The distribution and pattern of ICs deposited in mesangium and tubuli were examined with a confocal microscope.

Results: Mesangial ICs had typical irregular surface and variable size. Electron microscopy confirmed the distribution of ICs in glomeruli. Comparing data from confocal microscopy with results of routine immunofluorescence staining in pathology reports, glomerular IgG deposits were detected in 60% vs. 40% specimens (≥ 1+ intensity), respectively. Confocal microscopy showed tubular IgG and IgA deposits that were co-localized in 80% specimens, whereas 20% had neither immunoglobulin in tubuli. Aggregates of small ICs present in tubular lumen and adjacent to tubular epithelium represented the predominant pattern of the tubular ICs. Two biopsies showed cast-like tubular structures. All tubular ICs displayed IgA-IgG colocalization. Complement C3 was also co-localized in these ICs except in two biopsies. ELISA data confirmed presence of IgA-IgG ICs in all seven contemporaneously collected urine samples. Levels of urinary IgA-IgG ICs correlated with proteinuria, but not with the intensity of IgG staining in tubuli or glomeruli.

Conclusions: In summary, this study confirmed the presence of IgA-IgG ICs in renal tubules and in the urine of patients with IgAN.

1533 Fetal Ovine Congenital Obstructive Uropathy: Morphological Observations and Correlation with Metalloproteinase Activity

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Background: Congenital obstructive uropathy is the most common cause of renal failure in early childhood. Developing predictive tests for its occurrence and severity is important. The fetal lamb is an appropriate large animal model for these studies. Urinary metalloproteinase activity (MMP) has been found to be abnormal in these animal models. The aim of this study is to see if the profiles of MMP correlate with the degree of renal damage.

Design: Fetal lambs (N=32) were divided into 2 groups at 90-100 days gestation. Group 1 (N=15) underwent sham operation. Group 2 (N=17) underwent creation of complete urinary tract obstruction. Urinary MMP profiling was performed by gelatin zymography for 4 MMP species of 10 different molecular weights. Fibrotic change and degree of disorganization was assessed by semi-quantitative grading scale (0-4+) in subcapsular zone, labyrinth, medullary ray, outer medulla/inner stripe, inner medulla and pelvis. Medullary ray number per 10 mm was assessed. Statistical analysis was by Student t-test, Mann-Whitney U-test and multiple regression.

Results: No dysplastic changes were seen. Medullary ray number was reduced (6.2+/-0.9 vs 8.1+/-0.8) ($P<0.001$). The inner medulla was markedly fibrotic and attenuated with disorganization of remaining tubular structures in the obstructed animals. The most conspicuous fibrosis occurred in the inner medulla, subcapsular zone and pelvis. MMP-9 activity was a significant independent predictor of the total combined fibrosis score ($P<0.001$) and the fibrosis score in each of the individual areas analyzed ($P<0.01$). The activity of MMP-2 correlated only with inner medullary changes ($P=0.03$), inner stripe ($P=0.01$) and subcapsular fibrosis ($P=0.01$).

Conclusions: Urethral obstruction in the fetal lamb produces an altered renal cortical organization (reduction of medullary rays), fibrotic changes throughout the kidney and marked thinning and fibrosis of the inner medulla, without frank dysplastic changes. Urinary metalloproteinase activity closely correlated with these alterations and may be useful in assessing injury related to human fetal urinary obstruction.

1534 Correlation of Histologic Findings with Donor Specific Antibodies (DSA) in C4d Positive Renal Allograft Biopsies during the Early Post-Transplant Period

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Background: Peritubular capillary (PTC) deposition of C4d is considered to be immunopathologic evidence of antibody reactivity and one of the criteria for diagnosis of acute antibody mediated rejection. In cases without morphologic evidence of active rejection, the significance of C4d is unknown. In this study, we correlate histologic findings with presence of anti-HLA DSA in patients (pts) with PTC C4d.

Design: We searched our database for renal allograft bx that were obtained up to 3 months post-transplantation and showed diffuse staining of PTC for C4d, detected by either IF or IP. Sera were tested for DSA, and their antigenic specificity was recorded. Bx were grouped by histologic findings according to Banff criteria, and correlated with DSA.

Results: 32 pts met the entry criteria. The average time post-transplantation was 25 days (range, 7-70). The cases were divided into groups with: no significant changes (n=19), borderline lesions (n=3), ACR (n=8), and isolated TMA (n=2). The correlation between histologic findings and presence of DSA (Table 1), and histologic findings and DSA specificity (Table 2) is shown below.

Table 1

	NO TISSUE INJURY	TISSUE INJURY			
		BORDERLINE	ACR	TMA	ALL DX
	n (%)	n (%)	n (%)	n (%)	n (%)
DSA +	12 (63.2)	2 (66.67)	6 (75)	2 (100)	10 (76.9)
DSA -	7 (36.8)	1 (33.3)	2 (25)	0 (0)	3 (23.1)
TOTAL	19 (59.4)	3 (9.4)	8 (25)	2 (6.2)	13 (40.6)

Table 1

No tissue injury		Borderline		ACR		TMA	
class I	class II	class I	class II	class I	class II	class I	class II
CW02, B61	DR17		DQ02	A31		B60, CW10	
	DQ02		DR13		DR13		DR16,51, DQ05
B08,61	DQ02, DR11				DQ09		
	DQ9			CW01	DP4, 13		
	DR08, DQ04			A25, B07			
A32				A01	DQ07		
B08							
A03,24	DR01						
	DP0402						
A02	DQ02, PD0201						
A02							
	DQ07						

Conclusions: In our study 19/32 (59.4%) patients with PTC C4d did not have acute rejection or other graft pathology, and of these 7 (36.8%) did not have circulating DSA. In contrast, the great majority of pts with PTC C4d and tissue injury had circulating DSA (10/13, 76.9%). When DSA are identified, the groups with rejection/tissue injury appear to have different antigenic specificity than those without.

1535 Abnormal Pediatric Glomerular Basement Membranes – Novel Genetic Associations

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Background: Abnormal glomerular capillary basement membranes (CBM) are a feature of known genetic renal diseases such as Alport, nail-patella, and Pierson syndromes. We identified unclassifiable CBM lesions in 5 pediatric patients and sought to determine the etiologies.

Design: Records were reviewed for clinical findings and family histories, and all biopsies were assessed in the standard fashion. 4 biopsies additionally were assessed for colα3 and α5(IV), laminin α2, α5 and β2, podocin, synaptopodin, α-actinin-4, nephrin and WT-1 by IF and by PTA stain for EM. One biopsy (P1) had additional staining only for colα3 and α5(IV). CBM thickness was evaluated by EM morphometric measurement of the thickest and thinnest part of all non-oblique CBM between cell plasma membranes in at least 2 glomeruli.

Results: Age, proteinuria at presentation and morphologic findings are in the table below. All patients had normal renal function; patient (P) 1 had transient hypocomplementemia and peri-orbital edema, all others had normal serologies and no signficiant physical findings. Ultrastructurally, P1 had foci of thin CBM and thick CBM due to irregular intramembranous lucencies and slight layering and was found to be nephrin deficient. P2 and P3 showed similar findings with areas of thick and thin CBM with uniform electron density; P3 had absent α actinin 4 and a family history of focal sclerosis. P4 and P5 were similar wtih markedly thick CBM having a "moth eaten" pattern, segmental areas of entrapped cytoplasm, and coarsely scalloped subepithelial contours but without identified protein abnormalities.

Patient and Biopsy Features

Patient #	Age (yrs)	Proteinuria	Renal Family Hx	CBM Thickness in nm (Average)	Staining Results	Genetic Testing
1	2	Nephrotic	No	90-700 (280)	Colα3,α5(IV) normal	Absent nephrin
2	5	Pr/Cr 0.58	Yes	151-543 (305)	All normal	Not done
3	5	Pr/Cr 1.02	Yes	142-590 (323)	↓α actinin 4	Nephrin, WT-1, Lam-2 normal
4	8	Pr/Cr 0.94	Yes	266-2023 (886)	All normal	Not done
5	13	1.2 gms/ 24 hrs	Yes	516-2781 (1153)	All normal	Not done

Conclusions: Abnormal thick and thin CBM may be a manifestation of genetic forms of proteinuria such as α-actinin-4 or nephrin deficiencies. In patients with abnormal

CBM which cannot be classified into known renal diseases, podocyte genetic defects are a possible underlying cause. Therefore, patients with abnormal CBM of unknown etiology should be assessed for the known range of renal genetic deficiencies.

1536 The Pathology of Early Recurrent Membranous Glomerulonephritis (MGN)

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Background: MGN in the native kidney is characterized clinically by proteinuria and morphologically by subepithelial glomerular basement membrane (GBM) deposits of IgG and C3 by immunofluorescence (IF), subepithelial deposits visible by electron microscopy (EM), and diffuse podocyte foot process effacement (FPE). MGN may recur in the allograft and cause graft loss. Protocol and early indication biopsies allow detection of recurrence before it is clinically apparent. In this study we assessed the earliest morphologic manifestations of MGN in humans.

Design: Kidney transplant biopsy records from 2006-present at our institution were reviewed for cases of early recurrent MGN. Clinical data, IF reports, and EM were reviewed at the initial biopsy with evidence of recurrent MGN and on follow-up biopsies.

Results: Eight patients (F=5, M=3), were identified with early recurrent MGN on protocol (n=4) or indication (n=4) biopsy, with a total of 20 initial and follow-up biopsies. The mean age at the time of transplant was 48 yrs (range, 27-56 yrs). 7 patients were on routine maintenance triple immunosuppressive therapy; one was on a steroid-free protocol with tacrolimus and mycophenolate. The mean time of biopsy post-transplant with pathologic changes was 3.2 mos (range, 0.5-12; median 2.2). In each patient's earliest biopsy, IF showed granular GBM staining for C4d, IgG, and kappa and lambda light chains (8/8 cases). IF for C3 was negative (5/8) or showed trace segmental GBM staining (3/8). On each patient's earliest MGN biopsy positive by IF, 6/8 showed absence of detectable deposits by EM and 6/8 showed no significant podocyte FPE. Only 2/8 cases showed diffuse podocyte FPE; one showed small deposits by EM, and the other showed no deposits. Of the 6 patients that showed absent deposits by EM on the early biopsy, half (3/6) later developed tiny subepithelial deposits after a mean of 9 mos follow-up (range 6.7-13). At the time of the earliest MGN biopsy, the mean measured or predicted proteinuria was 0.94 g/day (range 0.17-2.3) in 7 patients without contaminating native kidney proteinuria.

Conclusions: Early recurrent MGN is characterized by C4d and IgG GBM deposits with absent or minimal C3, often no detectable deposits by EM and mild or no podocyte FPE, and minimal or mild proteinuria. Routine C4d staining in transplant biopsies may detect early recurrent MGN. Recognition of these unique features can aid in the diagnosis of early recurrent MGN before patients develop significant proteinuria.

1537 C1q Nephropathy in the Renal Allograft: A Report of 20 Cases

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Background: C1q nephropathy (C1qN) is an uncommon glomerular disease characterized by dominant or co-dominant mesangial staining for C1q in pts with no evidence of SLE. There are no series in the literature addressing the clinical and pathologic characteristics of pts with C1qN in the renal allograft.

Design: We identified and analyzed 20 cases of C1qN in renal allograft from our nephropathology archives, between 1998-2009.

Results: 80% of pts were whites and the M:F ratio was 1.5. The mean age at transplant was 30 yrs. The native disease was PCKD (35% of pts), FSGS (15%), HTN (10%), ANCA GN (10%), IgAN (10%), anti-GBM disease (5%), solitary kidney (5%), renal hypoplasia (5%) and oxalosis (5%). The kidney source was a living donor in 75% of pts and a deceased donor in 25% of pts. The mean time from transplant to diagnosis of C1qN was 44 mos (range 0.1-241 mos; >12 mos in 80% of pts). 45% of pts had preceding infection (most commonly urinary tract infection). None of the pts had clinical or laboratory evidence of SLE. The indication for biopsy was surveillance (60% of pts), worsening creatinine (Cr) (25%), proteinuria and worsening Cr (10%) and proteinuria (5%). At biopsy, 42% of pts had proteinuria which was > 1g/day in 21% of pts and mean Cr was 1.8 mg/dl. Only 11% of pts developed hematuria and none had hypoalbuminemia. One of the 3 pts whose native disease was FSGS had recurrent disease. The glomerular pattern on light microscopy was mesangial hypercellularity (45% of pts), FSGS (25%, including 1 of the 3 pts whose native disease was FSGS), and no lesions (30%). All cases showed intense (≥ 2+) dominant (65%) or co-dominant (35%) mesangial staining for C1q on immunofluorescence. Paramesangial electron dense deposits were seen in 14 of 17 (82%) biopsies studied ultrastructurally, but none showed subepithelial or subendothelial deposits or tubuloreticular inclusions. 5 pts were treated with pulse steroids for cellular rejection and 1 with plasmapheresis for recurrent FSGS. On follow-up (mean 20 mos), 35% of pts had stable Cr with no proteinuria, 12% had stable Cr with a reduction in proteinuria ≥50%, 29% had worsening Cr or <50% reduction in proteinuria, and 24% resumed HD or were re-transplanted (for causes other than C1qN).

Conclusions: C1qN in the renal allograft is a de novo disease in the majority of pts. It usually develops after the first year of transplant. Most cases are diagnosed as an incidental finding but some pts have mild proteinuria. Some cases may relate to infection. The most common histologic finding on light microscopy is mesangial hypercellularity.

1538 Collapsing Glomerulopathy (CG) in 13 Patients with Systemic Lupus Erythematosus (SLE): Cause or Coincidence?

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Background: CG is a primary podocytopathy characterized by segmental or global collapse of capillary walls with wrinkling of the glomerular basement membrane and overlying dedifferentiated podocyte proliferation. Idiopathic CG is a distinct clinicopathologic entity with poor response to immunosuppresive therapy and rapid progression to renal failure. CG has been associated with many etiologies including viral infections, autoimmune disease, and certain drugs. Although described in anecdotal case reports, we present the largest group of CG, thus far, in patients with SLE or SLE-like disease.

Design: Renal biopsies were studied from 13 patients with SLE (10) or SLE-like (3) disease diagnosed with CG. A retrospective analysis of clinicopathologic features and follow-up was done. Immunohistochemistry (IHC) for parvovirus and Ki67 were performed.

Results: The patients ranged from 16-63 years, M:F-2:11, and were predominantly of African descent (92%). At presentation, all patients had nephrotic syndrome with mean proteinuria (7g/24hr +/- 2.9), creatinine (Cr) 3 +/- 2.7 and BUN 44 +/- 37, except in one patient whose Cr/BUN was 26.5/155. The complement levels were normal (mean C3 105, C4 26). ANA was positive in 89%, anti-dsDNA 75%, and anti-Smith 25%. HIV, HCV, and Parvovirus serologies were negative in all patients tested. On morphology, globally collapsed glomeruli ranged from 1-52% (mean 20.7%), segmentally collapsed (0-20%), and globally sclerosed glomeruli (0-37%). Mild to severe tubular atrophy and interstitial fibrosis was seen in 35% with focal microcystic changes. Minimal glomerular mesangial deposits were seen by IF and EM in 57%, and extensive foot process effacement in 76%. Ki-67 positive epithelial nuclei were seen per affected glomeruli (mean 2.1). Parvovirus IHC in CG podocytes was positive in 4/11 patients. Initial treatment was pulse/oral steroids and dialysis (8). Follow-up from 9 patients showed 5 with end-stage renal disease, 0-21 months after biopsy;1 patient returned to normal cr and 3, 1.2-3.0mg/dl.

Conclusions: Immunologic (antibody or cytokine) mediated injury may lead to glomerular podocytopathy in SLE (minimal change disease, FSGS). However, other known causes of CG, having an impact on prognosis and therapy, should be considered in this setting. The high predominance of patients of African descent in our study suggests that podocytic injury and collapsing features could result from immunologic and/or environmental factors on a genetic predisposition to epithelial injury.

1539 Interobserver Agreement for the Polyomavirus Nephropathy (PVN) Classifying Schema in Renal Allografts

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Background: A classification schema for PVN grading was proposed at a 2009 Banff conference on allograft pathology. The schema introduced three stages of PVN: early (stage A), florid (stage B) and late sclerosing (stage C). In addition, histologic reporting of the viral load (VL) was proposed.

Design: To examine the applicability and the interobserver agreement for the PVN schema, we retrieved cases with confirmed PVN by histology and SV40 from our institutional database. We identified 24 allograft biopsies with PVN which fulfilled the adequacy criteria. Four pathologists independently scored the PVN stage (A, B or C), not knowing the clinical history. VL was scored as a numerical percent of tubules with viral replication and the results were assessed using either a 3-tier score (VL1: ≤1%, VL2: >1% to ≤10%, VL3: >10%) or a 4-tier score (VL1: ≤1%, VL2: >1% to ≤5%, VL3: >5% to 15%, VL4: >15%). Reviewer agreement on PVN stage was evaluated by Kappa statistic, whereas the agreement on VL was evaluated by the Kendall's coefficient of concordance. The type of our practice may be a potential study limitation, because cores are divided and processed in plastic and paraffin, with SV40 being performed only on paraffin cores.

Results: Complete agreement for the PVN stage score was found in 12 of 24 (50%) cases and 3 of 4 reviewers agreed in 8 of 24 (33%) cases. Complete disagreement for PVN stage was observed in 4 (17%) cases. The overall kappa score for PVN stage was 0.47 (95% confidence interval 0.35 - 0.60, $p<0.001$). The highest kappa value was found for stage A (0.66), followed by B (0.43) and C (0.24). Using the 3-tier VL score there was a complete agreement in 13 of 24 (54%) cases and 3 of 4 reviewers agreed in 7 of 24 (29%) cases. Using the 4-tier VL score, there was a complete agreement in 6 of 24 (25%) cases and 3 of 4 reviewers agreed in 10 of 24 (42%) cases. Overall, there was a substantial agreement between the reviewers using both the 3-tier and the 4-tier score for PVN VL (Kendall's concordance coefficients 0.72 and 0.76, respectively; $p<0.001$ for both).

Conclusions: This is a first attempt to evaluate the proposed PVN classifying schema. Moderate kappa agreement was achieved in scoring the PVN stage; reviewers however did not know the clinical history, which may have reduced the interobserver agreement. Although substantial agreement was found using both the 3-tier and the 4-tier VL score, a better complete agreement was found using the 3-tier score.

1540 Diagnostic Discrepancies between Frozen and Permanent Section Diagnoses in Pretransplant Donor Kidney Biopsies: Quality Assurance Study of 1887 Cases

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Background: Pretransplant donor kidney biopsies are mandatory when a donor kidney is the marginal category. In such cases, frozen section evaluation quickly provides useful information.

Design: Evaluation of donor kidney biopsy includes three histologic parameters (interstitial fibrosis; Ci, arteriosclerosis; Cv, arteriolosclerosis; Ah) scored as 0-3 according to Banff 97 criteria, counting the percentage of glomerulosclerosis (Gs), and describing additional pathologic findings such as glomerular abnormality, capillary thrombosis, infarct, acute tubular necrosis (ATN), and tumor. For this study, the types of, and reasons for, discrepancies between frozen and permanent section diagnoses were analyzed in 1887 pretransplant donor kidney biopsies obtained between March 1994 and April 2008. Discrepancies were then classified as major (difference of score ≥ 1

in Ci, Cv, or Ah and difference of percentage of Gs (≥ 20% GS vs < 20 % GS)), minor (difference of score < 1 in Ci, Cv, or Ah) and miscellaneous (capillary thrombosis, glomerular abnormality, ATN, infarct, and tumor).
Results: 157 kidneys among 1887 pretransplant donor kidney biopsies (8.3%) showed discrepancies between frozen and permanent section diagnoses. 78/165 discrepancies were considered major (47.3 %), 68/165 minor (41.2%), and 19/165 miscellaneous (11.5 %). The most frequent major discrepancies were Ah (35.9%) and Gs (35.9%), followed by Cv (21.8 %) and Ci (5.1%). Minor discrepancies included Cv (47.1 %), Ah (27.9 %) and Ci (25.0 %). Miscellaneous discrepancies included glomerulopathy (78.9 %), capillary thrombosis (10.5 %), and ATN (10.5 %). Most Ah lesions were underestimated (87.2 %) in frozen section. All the Gs-related discrepancies were attributable to sampling variability (100 %). Cv was falsely estimated (overestimate; 49.0 %, underestimate; 51.0 %) most often attributed to sampling variability. Ci was often overestimated (71.4 %) mainly due to misinterpretation of interstitial edema. Most glomerular abnormality-related discrepancies were due to underestimation of Kimmelstiel-Wilson lesions (93.3 %).
Conclusions: Major discrepancies between frozen and permanent section evaluation of donor biopsies is uncommon, but not rare and most often includes underestimation of Ah, overestimation of Ci, and sampling variability in Gs and Cv scoring. Surgical pathologists should be aware of these potential pitfalls and limitations for an accurate evaluation of pretransplant donor kidneys.

1541 Discrepancies in IgG Subtype Composition between Glomerular and Tubulointerstitial Immune Complex Deposits in Proliferative Lupus Nephritis

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Background: Tubulointerstitial immune complex deposition is a histologic characteristic of lupus nephritis. However, the degree of tubular basement membrane (TBM) immune complex deposition does not seem to correlate well with glomerular immune complex deposition. We suspect that TBM immune complex deposits differ from glomerular deposits. We studied if there is any correlation in the IgG subtype staining between glomerular and TBM immune complex deposits and also if presence/ absence of interstitial inflammation correlated with any IgG subtype predominance in the TBM deposits.
Design: Native renal biopsies with lupus nephritis from 60 patients were stained with antibodies to the IgG subtypes by direct immunofluorescence (IF). TBM deposits were present in 39 of the biopsies. Staining was semiquantitatively scored as 0, trace, 1+ (mild), 2+ (moderate) and 3+ (prominent).
Results: IgG1, IgG2 and IgG3 were the predominant subtypes in the glomerular immune complex deposits. The tubulointerstitial deposits followed this pattern but with a milder intensity. However, in nine biopsies, the predominant IgG subtype composition in the TBM deposits differed from that in the glomerular deposits.

Predominant IgG subtype composition in glomerular and tubular basement membrane immune complex deposits in biopsies with proliferative lupus nephritis.

Case Number	Glomerular	TBM	Inflammation
1	1	3	absent
2	1,3	1	absent
3	1,2,3	2,3	absent
4	1,2,3	1	present
5	1,3	2,3	absent
6	1,2,3	3	present
7	1,2,3	1	present
8	1,3	1	absent
9	1,2,3,4	1,2	absent

TBM=tubular basement membrane

Therefore it appears that, in native kidney biopsies with lupus nephritis, the pattern of IgG subtype staining in tubulointerstitial deposits does not always correlate with that seen in glomerular deposits. Six of these biopsies showed no interstitial inflammation, three biopsies showed varying degrees of interstitial inflammation. The presence/ absence of interstitial inflammation did not appear to correlate with the IgG subtype composition of the TBM deposits. All of these nine biopsies with discrepant TBM IgG subtype composition were cases of proliferative lupus nephritis with or without membranous component. Cases of purely membranous lupus nephritis were not considered because overall they showed very little to none TBM staining.
Conclusions: Based on our data, it appears that the pathogenesis of TBM deposits is different than that of glomerular deposits.

1542 Pathogenesis of Minimal to Mild Diabetic Glomerulopathy

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Background: Textbooks say that (1) the main mesangial destructive lesions in diabetic glomerulopathy are the diffuse lesion (DL) and the nodular lesion (NL), (2) the DL is far more common and more highly associated with renal insufficiency than the NL, and (3) the NL never develops in the absence of significant DL. These conclusions were based mostly on the study of advanced lesions. As far as I know, the pathogenesis of minimal to mild diabetic glomerulopathy has not been determined.
Design: Seventy four diabetic (end stage cases were excluded) and 59 matched control cases were retrieved from consecutive UTMB autopsies. Twenty five cases had minimal to marked mesangial lesions. About 100 glomeruli from each of these 25 cases were studied using 18 4 micron paraffin embedded serial sections stained as follows: PAS, IgG, IgM, PAM, Trichrome, IgA, Albumin, Lysozyme, PAS, Fibrinogen, Fibronectin, PAM, Trichrome, Kappa and Lambda light chains, LCA, PAS and Actin. Starting at PAS-9, NLs, focal mesangiolyses (FMs) and non NL peripheral mesangial expansions (PMEs) > 40 microns were traced through the serial sections, noting their dimensions and other characteristics. PMEs between 15 and 40 microns were counted, and the DL graded on a scale of 0-5+ (0 is normal, 5+ is 5 times normal) in the same glomeruli in PAS-9 only.
Results: NLs, FMs and FMEs always occurred at the periphery of lobules. Ten cases with the least number of these lesions are presented here (7 males and 3 females, mean ages 58 and 69, respectively, mean duration of diabetes 18 yrs, 9 type II and 1 type I diabetes). Mean number of traced glomeruli with lesions was 9.2 (4-15) in PAS-9. Mean number of total lesions (NLs, FMs and PMEs >40 microns) in traced glomeruli in all sections was 15.9 (0-38), 4.3 (0-11) and 5.4 (0-15), respectively. Mean number of PAS-9 glomeruli with PMEs 15-40 microns was 14 (5-31), and mean number of these smaller PMEs was 18.2 (5-35). Two cases had + DL, 4 had ++ DL and 4 had ++variable DL (>++ in afferent primary branches, but <++ in predominantly efferent areas. DL grade ++ would be considered mild by most renal pathologists, but sufficient to suggest diabetes if at least 20 glomeruli were available. Most lesions were modest in size, but 4 cases had a total of 3 laminated NLs up to 80x80 microns and 3 FMs up to 80x100 microns. Another case with + DL had 2 large laminated NLs, 1 with an FM and 1 with a microaneurysm 70x80 microns.
Conclusions: The findings in these 10 cases indicate that destructive mesangial lesions all occurred at the periphery of lobules, and developed in the presence of minimal to mild DL.

1543 The Fate of Renal Allografts in Patients with Donor Specific Antibodies

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Background: Antibodies to donor HLA antigens (DSAs) are a feature of the allogenic immune response to a renal transplant. Despite the growing awareness of antibody-mediated rejection, the relationship between DSAs and both acute and chronic allograft dysfunction needs to be better defined. To this end, the present study is a retrospective analysis from our institution of the fate of all kidney transplant recipients in whom DSAs were detected.
Design: All patients (1981-2007) were screened post-transplantation for Class I DSAs, whereas Class II screening began in 2003 with the appearance of solid state technology. Diagnoses of acute antibody-mediated rejection (AMR), acute cellular rejection (AR), or chronic, active T cell-mediated rejection (CR) were based on the Banff '05 criteria (applied retrospectively to biopsies prior to 2007).
Results: 76 patients with DSAs had obtainable records. 6 had a nephrectomy within 1 month for surgical complications and were not studied. 39 patients had 2 or more transplants. Of these, 9 transplants had DSAs. Thus, 70 patients with 79 renal allografts developed DSAs (42 males and 37 females). Class I antibodies (Abs) developed in 67 transplants. Class II Abs were present in 33 of 52 transplants screened, and both Class I and II in 21. Acute antibody-mediated rejection occurred in 8 (10%). All had Class I Abs. Of the 4 patients screened, 2 also had Class II Abs. Acute cellular rejection occurred in 31 (39%). 16 had Class I Abs. Of the 22 patients screened, 4 had Class II Abs alone and 11 Class I and II Abs. 8 class I patients had A2, 6 A1, and 4 B44 Abs. 3 Class II patients each had DQ2, DR1, or DR15 Abs, respectively. Chronic, active T cell-mediated rejection was diagnosed in 45 (60%) (the mean time to CR was 18 months). Of these 45, 22 did not have a previous episode of AMR or AR. 5 previously had AMR and 18 AR. Fifty-two allografts of the 79 allografts (66%) required a nephrectomy (mean time to surgery 24 months). 6 transplants experienced acute tubular damage without evidence of AMR, AR, or CR.
Conclusions: Over 75% of patients with DSAs experienced some form of rejection (AMR, AR, and/or CR (60%)) with a mean for CR of 18 months following transplantation. Almost 70% required a transplant nephrectomy within a mean of 2 years. Our data clearly indicate that patients with DSAs fared quite poorly. In general, the appearance of DSAs in a renal transplant recipient indicates the likelihood of a strong anti-allograft diathesis.

1544 Evaluation of Non-Neoplastic Parenchyma of Nephrectomies with Neoplasms

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Background: Evaluation of non-neoplastic parenchyma of kidneys resected for neoplasms provides important information concerning the presence and severity of concomitant medical renal diseases. As these data may have considerable impact on patient management and prognosis, competent and accurate assessment of the non-neoplastic specimen is necessary. Indeed, the College of American Pathologists has recently required this for protocols for renal cancers. We report our experience in a large series of such nephrectomies.
Design: 311 consecutive nephrectomies/nephroureterectomies performed in 2005-2008 were evaluated. Non-neoplastic parenchyma at a distance from the mass was examined with thin sections stained with hematoxylin and eosin, periodic acid-Schiff, periodic acid-methenamine and Masson trichrome stains; these were supplemented with other stains and/or electron microscopy when indicated. The findings were correlated with relevant clinical information available at the time of surgery.
Results: Patient ages ranged from 14-95 (median 65) years with male:female 1.6:1. 95% had abnormalities, the majority (66%) with nephrosclerosis; 41% of these (24% of the total) were considered mild. Other important lesions included chronic interstitial nephritis with features of obstruction (8.4%), diabetic nephropathy (7.4%), focal segmental glomerulosclerosis (4.8%), acquired cystic disease (5.5%) and miscellaneous (glomerulonephritis, atheroemboli, amyloid, acute tubular injury, etc 3.9%). In only few instances (3%) were these diagnoses indicative of unknown diseases.
Conclusions: Kidneys with neoplasms often harbor medical diseases which require identification and/or assessment of degree for prognostication and for possible therapies beyond those designed for the tumor. Careful pathologic examination of the non-

neoplastic parenchyma is necessary as these diseases may represent important causes of morbidity and mortality in this era of effective neoplasm management.

1545 Glycoportein nmb (Gpnmb) Is Upregulated with Injury in the Rat 5/6 Nephrectomy (Nx) Remnant Kidney (RK)

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Background: Gpnmb,a transmembrane glycoprotein, influences osteoblast differentiation, cell adhesion and cell migration. It is found in the renal tubulo-interstitium in an obstruction model and is increased in circulating monocytes from end stage renal disease patients. We identified markedly increased Gpnmb mRNA expression in 5/6 Nx RK using microarray with Affymetrix chip 230_2 and sought to confirm the expression and localization of Gpnmb in this model, as well as assess downstream activation of the cSrc-Syk signaling pathway, which regulates autophagy and resultant tubule cell survival or death.

Design: Rat 5/6 Nx RK and control kidneys (C) were stained by immunohistochemistry for Gpnmb, macrophages (F4/80), WT-1 and active phospho-Syk, and with lectins using Peanut agglutinin (PNA) and Phytogemagglutinin E (PHA-E) for tubular localization.

Results: Gpnmb staining in the cortex and medulla is detailed in the table below; there was no proximal tubular staining. At 2 and 4 wk, RK cortical distal and medullary tubules often were dilated and frequently contained Gpnmb+ cellular debris. In the 2 wk RK, phospho-Syk is upregulated in cortical distal tubular cells and occasionally co-localized with Gpnmb in the apical areas. Glomerular Gpnmb was found in capillary luminal macrophages and in areas of injury was associated with loss of WT-1 podocyte staining.

Gpnmb Kidney Staining

	Control	RK 2D	RK 2 Wk	RK 4Wk
Cortical Distal Tubular Cells	Focal apical	↑apical, mild diffuse	↑ extent, intensity	↑ extent, intensity
Medullary Tubular cells	Extensive, weak-moderate apical, often diffuse	No change	No change	No change
Glomeruli	Negative	Focal segmental capillary	Focal segmental in injured foci, capillaries	Focal segmental in injured foci, capillaries
Infarcted tissue	Not present	+ macrophages	+ macrophages	+ macrophages

Conclusions: Gpnmb is upregulated in viable renal tubular cells over time in the 5/6 Nx RK. The co-localization of Gpnmb with Syk phosphorylation provides evidence supporting the presence of an activated Gpnmb(ITAM)-cSrc-Syk signaling pathway during renal injury. The segmental loss of WT-1 glomerular staining associated with Gpnmb suggests there is a link to podocyte dedifferentiation. Gpnmb, a protein found to play a role in lysosomal trafficking and autophagy, also may play a pathogenetic role as renal injury advances, and may be a useful biomarker for progressive kidney injury.

1546 p53 Immunostaining Can Be Used as a Sensitive Measure to Detect BK Viral Infection in Renal Allograft Biopsies

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Background: New Banff criteria have been proposed to stratify BK virus infection in renal transplants into stage I (early focal), stage II (extensive) and stage III (late sclerosing). This study was performed to determine if p53 can be used as a sensitive marker to assist in diagnosing BK virus infection in the renal allograft biopsy.

Design: Renal transplant biopsies from both control group (n =16, no BK virus infection) and BK group (n = 13, with known positive BK virus in urine by PCR tests) were immunohistochemically stained for BK virus (SV40) and p53. The nuclear staining intensity was graded on a scale of 0 to 3+ and positive percent involvement in the renal parenchyma was recorded.

Results: Staining intensity. None of the biopsies from the control group was positive for BK virus staining (0/16). 13/16 cases showed weak (1+) to moderate (2+), fine granular, complete nuclear staining for p53 (reactive epithelial changes without nuclear atypia). 3/16 cases were negative for p53 staining, using paraffin embedded tissue. In the BK group, 9/13 cases stained strongly positive (2-3+) and 3/13 stained weakly positive (1+) for BK virus. 12/13 cases in the BK group showed strong (3+) p53 nuclear staining with nuclear translucent halos. Positive staining percent. 8/13 cases of the BK group showed higher percent of p53 positive staining (10 to 30%) when compared to BK positive staining (1 to 5%). In 3/13 cases of subtle BK infection, focal BK infection was confirmed by a unique pattern of strong p53 staining with nuclear halos. Two (2/13) non-conclusive cases in BK group. The first nonconclusive case (non-conclusive #1) was that of a small biopsy sample that stained negatively for BK virus but focal strongly positive for p53 by immunohistochemistry, but was positive for BK virus in the urine cytology within 1 week. The other non-conclusive case (non-conclusive #2) showed weak BK staining in two tubular cells that had normal nuclear size, but p53 staining was negative. This case was called suspicious for early BK infection.

Conclusions: Our data suggest that the unique pattern of p53 staining (with nuclear halos) may be useful to assist in confirming the presence of subtle BK virus infection (stage I) or more accurately estimating the extent of BK virus infection in the renal allograft biopsy (stage II).

1547 A 25-Year Institutional Experience with Anti-Glomerular Basement Membrane Disease in Pediatric Renal Biopsy and Autopsy Specimens

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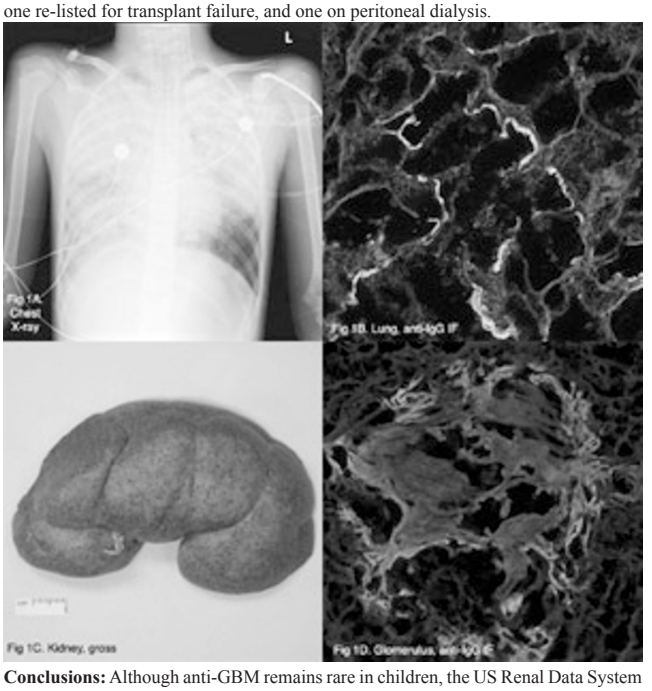
Background: Anti-glomerular basement membrane (anti-GBM) disease is characterized by rapidly progressive glomerulonephritis (RPGN) and autoantibody formation against type IV collagen in the GBM. As antibodies may react with alveolar basement membrane, pulmonary hemorrhage is the third component in Goodpasture Syndrome. Exceedingly rare in children, mean age of onset is 20-30 years with a second peak at 50-70. In adults, the pulmonary component is associated with cigarette smoking and inhaled hydrocarbon vapor, although the mechanism is unclear.

Design: We reviewed renal biopsy and autopsy specimens over 25 years from a large US children's hospital, basing the diagnosis of anti-GBM disease on clinical RPGN, crescentic glomerulonephritis by light microscopy, and linear immunofluorescent (IF) IgG staining of the GBM in patients 18 or younger, correlating with serum findings.

Results: We identified 3 cases by renal biopsy and 1 at autopsy.

	Gender	Age	IgG IF	Serum anti-GBM	ANCA	Pulm. Hemorrhage
1	F	8	+	-	+	+
2	M	10	NA	+	NA	+
3	M	17	+	+	-	+/-
4	M	10	+	+	-	-

Two patients presented with pulmonary complaints and had pulmonary hemorrhage during admission, neither with known inhaled exposure. Remote hemoptysis with smoking was present for patient 3, while a fourth had exposure to engine exhaust and renal symptoms only. Interestingly, the autopsy case (1) showed + IgG IF despite undetectable serum anti-GBM level and + serum ANCA (Fig 1). The 3 surviving patients continue to do well (6 months - 18 years later), one after successful renal transplant, one re-listed for transplant failure, and one on peritoneal dialysis.



Conclusions: Although anti-GBM remains rare in children, the US Renal Data System suggests that more cases occur than previously reported. Our sample size is small, but the association with inhaled agents may exist for children as well as adults. Serum anti-GBM antibody is typically present, but adult cases with undetectable levels have been recently described. Some patients are both anti-GBM and ANCA +, with a small subset ANCA +, anti-GBM -. To our knowledge, ours is the first such described pediatric case.

1548 IgA-Dominant Postinfectious Glomerulonephritis (IgA-PIGN) Is Frequent in Non-Diabetic Patients with Staphylococcus Aureus (SA) Infection

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Background: IgA-PIGN was first associated with methicillin-resistant *Staphylococcus aureus* (MRSA) infections in studies from Japan. Recent North American studies found an association with diabetic nephropathy, but it was also documented in non-diabetic patients.

Design: We identified 6 patients with dominant or co-dominant IgA-PIGN, representing 1% of 608 renal biopsies examined in our institution from 07/2007 to 08/2009, in a population-based practice. We reviewed the clinical presentation, the biopsy and microbiology findings, and we obtained patient follow-up.

Results: All patients (4 males, 2 females) presented with acute renal failure (creatinine range, 527-1086 µmol/L), hematuria, proteinuria and hypertension. Median age was 62 years (range, 46 to 86). Only one patient had diabetes mellitus, but no biopsy-proven diabetic nephropathy. Complements C3 and C4 were normal in 4 of 5 patients with available data; low C3 and C4 were seen in one patient each. All 6 patients had documented infections at the time of the biopsy. Three of 6 patients had SA infections confirmed within two weeks before renal biopsy; two were MRSA. CMRSA-10 (equivalent to USA-300), clone responsible for the ongoing communitywide MRSA epidemic was identified in one patient. On biopsy, diffuse proliferative endocapillary GN was found in 5 patients and membranoproliferative GN in one. Diffuse proliferative crescentic GN was seen only in one patient, but no crescents were found in the other patients. On immunofluorescence, IgA subepithelial and mesangial immune complexes were dominant in 4 patients and co-dominant with IgG in the remaining 2 patients. On electron microscopy, large subepithelial deposits ("humps") measuring from 360-4100 nm, were found in all patients. Temporary hemodialysis was performed in 5 of 6 patients; only the diabetic patient remained on dialysis after 6 months. All non-diabetic

patients improved their renal function (creatinine range, 99-155 μmol/L; mean follow-up 6 months). Creatinine however remained well above the previous baseline range of 65-77 μmol/L.
Conclusions: IgA-PIGN was mostly found in non-diabetic patients in this study, in contrast with some previous studies showing strong association with diabetic nephropathy. We confirm that IgA-PIGN is often associated with SA and MRSA infections and for the first time we found an association with CMRSA-10. Although renal function improved in all non-diabetic patients, full recovery was not seen in any of the patients during the follow-up.

1549 Genetic Modulation of Anti-Myeloperoxidase Induced Murine Crescentic Glomerulonephritis

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Background: Anti-neutrophil cytoplasmic autoantibodies (ANCA), including anti-myeloperoxidase (MPO), are associated with crescentic glomerulonephritis (GN). Similar GN is induced in mice by injecting anti-MPO IgG. Patients have a spectrum of ANCA GN severity, whereas the inbred mouse strain that was initially used as a model (C57BL/6) has a narrow range of severity. The experiments reported here demonstrate that anti-MPO IgG causes GN of different severity in genetically different mice that is regulated primarily by differences in leukocyte responsivity to anti-MPO.
Design: Crescentic glomerulonephritis was induced in mice by iv injection of 50 μg/g anti-MPO IgG. Mice were sacrificed on day 6. Anti-MPO was given to: 1) 129S6 mice and C57BL/6 (B6) mice, 2) F1 mice from B6 x 129S6 mice, 3) F2 mice generated by B6 x 129S6 F1 intercross. 4) 129S1 mice, 5) chimeric mice with genetic differences between 129S6 or B6 donor bone marrow (BM) . To test the functional effects of genetically determined differences in disease severity, in vitro activation by anti-MPO IgG was evaluated in neutrophils from B6, 129S6 and 129S mice. Neutrophils were primed with 10ng/ml TNFa, stimulated with anti-MPO IgG, and superoxide generation measured.
Results: After injection of anti-MPO IgG: 1) B6 mice (n=17) developed an average 9% crescents, whereas 129S6 mice (n=13) developed 69% crescents. 2) F1 (B6 x 129S6) mice developed 13.5% crescents. 3) Of 60 F2 mice, 30 had < 10%, 24 10-25 %, 5 26-50% and 1 >50% crescents. 4) 129S1 developed 21% crescents and thus had less disease than 129S6 mice. 5) Rag2-/- B6 mice (n=4) that received BM from 129S6 mice had 79% crescents similar to 129S6 mice; however, Rag2-/-129S6 mice (n=6) that received BM from B6 mice had 17% crescents more similar to B6 mice. In the functional assays, anti-MPO IgG caused more activation of neutrophils from 129S6 mice than neutrophils from B6 or 129S1 mice.
Conclusions: 1) pathogenic events in this model of ANCA disease are influenced by genetic factors, 2) this genetic influence acts primarily through modulation of the responsivity of bone marrow derived cells (e.g. neutrophils) to activation by ANCA, 3) identification of the genes responsible for this modulation should reveal genes and gene products that have important roles in pathogenesis of ANCA disease, and could be markers of disease activity and outcome, as well as targets for therapy.

1550 Phosphorylated Mammalian Target of Rapamycin Is Upregulated in Both Cytoplasm and Nuclei along Cystic Epithelium of Adult Type Polycystic Kidney Disease

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Background: Mammalian target of rapamycin pathway (mTOR) is a dominant growth pathway in development of polycystic kidney disease (PKD) in both animal models and human adults, since the mutated polycystin-1 gene activated this pathway (PNAS 2006; 103:5466-5471). It is known that phosphorylated form of mTOR complexes with either raptor (to form mTORC1 for activating its downstream signals p70S6K and elF4E to stimulate cell proliferation) or rictor (to form mTORC2, a rapamycin-insensitive complex, for mediating its downstream targets including PKCα). mTORC1 mainly stays in the cytoplasm and mTORC2 is chiefly located in nuclei. In this study, we stained p-mTOR and one of its main down stream signals p-p70S6K in adult type PKD (autosomal dominant) and compared the stain intensity with unremarkable renal tubules.
Design: 10 cases of human adult type of PKD and 10 cases of unremarkable renal parenchyma (from nephrectomy for tumors) were stained for p-mTOR (Ser 2448) and p-p70S6K (Thr 389) (from Cell Signaling Technology), using a Dako Autostainer. Chromogenic cytoplasmic and nuclear staining was assessed and categorized into four grades: 0 (background), 1+ (weak), 2+ (moderate), or 3+ (strong) based on intensity of staining.
Results: In our recent study, we found that all cystic lining stained positively for cytokeratin 7, confirming that the cystic lining of PKD was originated from the distal nephron tubules. In all cases (10/10) of the current study, p-mTOR showed strong (3+) staining in both nuclear and cytoplasmic cellular compartments of both residual distal nephron tubules and cystic lining compared to moderate (2+) cytoplasmic staining of p-mTOR in the normal distal nephron tubules without significant nuclear staining. However, the p-p70S6K showed moderate (2+) nuclear intensity in approximal 50% of cystic lining, similar to the distal nephron tubular epithelium.
Conclusions: In the cystic epithelium and residual distal nephron tubules of PKD, upregulated p-mTOR in both cytoplasm and nuclei may enhance formation of both mTORC1 and mTORC2 . Unchanged expression of p-p70S6K of mTORC1 branch in cystic epithelium implies that p-p70S6K may only partially contribute to cystic formation, whereas the other branch of mTORC1 via elF4E is still under investigation. In contrast, mTORC2 branch may play a major role in the cystic formation of PKD.

1551 The Balance of Thymosin β4 and Its Metabolite Ac-SDKP Modulates Activity of Profibrotic Factors

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Background: We have previously shown that the G-actin sequestering protein thymosin β4 (Tβ4) is dramatically upregulated in the obstructed kidney in the unilateral ureteral obstruction (UUO) model of tubulointerstitial fibrosis. Tβ4, which is postulated to have profibrotic effects, is degraded by prolyl oligopeptidase (POP) to Ac-SDKP, a peptide with anti-fibrotic actions. Our previous study further revealed that inhibition of POP altered the balance of Tβ4 and Ac-SDKP and exacerbated fibrosis in obstructed kidneys. We now investigated whether the balance of Tβ4 vs Ac-SDKP also affects kidneys without injury.
Design: Adult male C57BL/6 wild type mice underwent UUO and were divided into five groups: UUO without treatment, UUO+POP inhibitor (S17092, 40mg/kg per day, by gavage), UUO+Tβ4 (150μg/d, i.p.), UUO+combination (POP inhibitor and Tβ4), and UUO+Ac-SDKP (1.6 mg/kg/d, delivered by minipump, starting 5 days before the surgery). Mice were sacrificed 5 days after UUO and the contralateral non-injured kidneys were studied.
Results: POP activity was significantly lower in the contralateral kidneys of mice treated with POP inhibitor, combination or Ac-SDKP (POP inhibitor, 12.7±1.3; combination, 18.3±1.4; Ac-SDKP 25.4±2.6; vs untreated UUO, 40.1±3.0 pmol/min*mg tissue, all $p<0.05$). The Ac-SDKP level in the contralateral kidneys was remarkably increased only in the group receiving Ac-SDKP (Ac-SDKP, 4.06±0.34; untreated UUO, 2.14±0.30 pmol/mg tissue, $p<0.05$). Compared to untreated UUO, plasminogen activator inhibitor (PAI-1) and Tβ4 expression in the contralateral kidneys assessed by real time PCR were dramatically reduced by Ac-SDKP treatment, but not affected by POP inhibitor with or without Tβ4. There was no difference in transforming growth factor (TGF)-β1 mRNA among these five groups.
Conclusions: Our study suggests that exogenous Ac-SDKP may have negative feedback to decrease POP enzyme activity, thus potentially decreasing endogenous Ac-SDKP production. However, in the non-injured contralateral kidney, exogenous administration of Ac-SDKP inhibits profibrotic factors. We propose that Ac-SDKP is a crucial molecule in determining fibrosis.

Liver & Pancreas

1552 Looking for Recipient Cells in Donor Tissue: Do Recipient Cells Transform To Become Hepatocytes in Allograft Liver?

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Background: If the recipient uncommitted stem cells were shown to assist with regeneration/repopulation of hepatocytes post-transplant, some marginal organs would be more easily acceptable as a solution to organ shortage. Studies from bone marrow transplant patients, although inconclusive, suggest donor cells differentiate to mature hepatocytes, but no evidence exists that recipient bone marrow cells contribute to liver allograft hepatocellular regeneration. This study aims at investigating this concept using Fluorescent in-situ hybridization (FISH) methods.
Design: Male-female mismatched recipients were identified in the Toronto General Hospital liver transplant record. FISH staining was performed on the most recent post-transplant biopsy for X-Y chromosome markers. HepPar-1 staining was superimposed on FISH to identify hepatocytes. Working on the concept that X/Y-carrying hepatocytes in male recipients of female organ implies contribution by recipient cells in post-transplant hepatocyte repopulation, 200-500 hepatocytes were evaluated per specimen.
Results: Study set includes native liver biopsies, one each from one male (N-M) and one female (N-F) patients; 9 allograft biopsies from male recipients of female (whole or split liver) organs (F-M); and one allograft biopsy from a female recipient of female organ (F-F). The native biopsies confirm specificity of the FISH method, F-N showing only X chromosomes while M-N shows both X and Y chromosomes in hepatocytes. The studied F-M and F-F biopsies were performed 6-102 months post-transplant, and all show only X chromosomes in hepatocytes confirming donor origin. Non-hepatocyte cells (HepPar-1 negative) however show both X and Y chromosomes in M-F samples.