

of gliotoxin on A-T, normal, and A-T cells expressing recombinant ATM to examine the role of ATM in gliotoxin resistance.

Design: A-T, normal, and A-T cells expressing recombinant ATM were treated with gliotoxin and the effect on colony-forming efficiency, ATM kinase activity, and dsDNA break formation was examined. Additionally, the effect of quercetin and epigallocatechin gallate treatment (flavonoids found in vegetables and green tea, respectively) was examined on cell colony-forming efficiency, dsDNA break formation, and ATM activation, both with and without later gliotoxin exposure.

Results: We found that; 1) ATM expression in A-T cells increased colony-forming efficiency following gliotoxin exposure, 2) gliotoxin activated ATM kinase activity, and 3) gliotoxin preferentially induced dsDNA breaks in A-T cells compare to normal cells and A-T cells expressing recombinant ATM. Additionally, pretreatment of normal cells with quercetin and epigallocatechin gallate increased cell gliotoxin resistance, and suppressed gliotoxin-induced dsDNA breaks. Similar results were obtained with A-T cells, though to a lesser extent. Interestingly, low flavonoids concentrations did not inhibit cellular colony-forming efficiency or cause dsDNA breaks, but did activate ATM in normal cells.

Conclusions: We demonstrate that ATM expression confers gliotoxin resistance. Additionally, two common dietary flavonoids confer gliotoxin resistance in normal and A-T cells and activate ATM in normal cells, demonstrating that these flavonoids increase cellular gliotoxin resistance via both ATM-dependent and -independent mechanisms. Thus; 1) ATM expression confers gliotoxin resistance, 2) gliotoxin and two nontoxic flavonoids activate ATM, and 3) pharmacological activation of ATM via flavonoid intake may prove useful in the treatment of aspergillosis via lessening gliotoxin toxicity.

1194 Comparison of Immunofluorescence Antibody Testing and Two Enzyme Immunoassays in the Serologic Diagnosis of Malaria

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Background: Serologic testing in malaria may be utilized to aid in cases of low parasitemia, as a screening tool for blood banks, and in the retrospective diagnosis of malaria in a previously non-immune individual. It has traditionally been done by immunofluorescence antibody testing (IFA), but the use of commercially available enzyme immunoassays (EIA) has become more widespread.

Design: We compared IFA with two commercial EIA kits, the Cellabs Pan Malaria CELISA and the Newmarket Malaria EIA. Seventy-five samples from 74 patients with clinically suspected malaria were examined by both EIA kits. The samples were also examined by IFA (n=48) and/or Giemsa stained blood smear (n=48). Fifty healthy blood donor samples, 11 rheumatoid factor (RF) positive samples, and 11 anti-nuclear antibody (ANA) samples were also examined by the two EIA kits.

Results: Using a consensus result as the gold standard, the agreement, sensitivity, and specificity with 95% confidence intervals were, respectively: Cellabs EIA 93.2%, 95.5% (82.7 - 99.2%), and 92.2% (86.7 - 93.8%); Newmarket EIA 87.7%, 68.2% (54.1 - 74.6%), and 96.1% (90.0 - 98.8%); and IFA 89.1%, 86.4% (73.7 - 92.4%), and 91.7% (80.1 - 97.2%). Compared to positive Giemsa stained smears, the sensitivities were: Cellabs EIA 90.9% (10/11); Newmarket EIA 54.5% (6/11); and IFA 100% (11/11). ANA positive sera (n=11) and RF positive sera (n=11) showed no cross-reactivity with the Newmarket EIA, while the Cellabs EIA yielded positive results in one ANA positive and two RF positive sera. Among healthy blood donors (n=50), the Newmarket EIA showed 100% specificity (50/50) and the Cellabs EIA showed a specificity of 92% (46/50).

Conclusions: We conclude that while the Newmarket EIA was a generally more specific assay, it was insufficiently sensitive relative to the IFA and the Cellabs EIA for screening purposes for malaria antibodies. The Cellabs EIA demonstrated the best overall sensitivity and is a reasonable choice as a serodiagnostic tool for malaria. It may also be useful as an adjunct to Giemsa stained smear examination, to aid in cases of low parasitemia in previously non-immune individuals.

1195 Comparison of the Neutrophil Volume Distribution Width with Manual Band Count, C-Reactive Protein and Absolute Neutrophil Count for Predicting Acute Infection

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Background: The accurate diagnosis of acute infection is very important for proper patient management. Laboratory tests commonly ordered include complete blood count with differential, absolute neutrophil count (ANC), manual band counts, C-reactive protein (CRP) and blood culture. We have previously demonstrated that neutrophil volume distribution width (NDW) was significantly increased in bacteremic patients compared to controls. The NDW, which reflects neutrophil size variability, is quantitatively determined by Coulter hematology analyzer (Coulter LH750) with VCS technology during automated differentials. In this study, we compared the NDW to other laboratory parameters, such as manual band counts, CRP and ANC for predicting acute infection.

Design: We retrospectively analyzed data from 140 patients (M/F = 84/56; mean age = 50 years), who were subdivided, based on the medical history chart review, into three groups: group 1 (N = 27), no clinical evidence of infection; group 2 (N = 46), localized infection; group 3 (N = 67), severe or systemic infection. The data included the percent band count, CRP, ANC and the NDW generated by Coulter LH750. Statistical analyses were performed using ANOVA, Pearson correlation and ROC methods.

Results: The NDW (p<0.001), CRP (p<0.05), the band counts (p<0.001) and the ANC (p<0.001) were significantly increased in groups 2 and 3 compared to group 1. There were good correlations between the NDW and band counts as well as ANC (r = 0.3; p<0.05 and r = 0.6; p<0.001, respectively) in the severely infected patients. ROC analyses revealed that the NDW was the best with an area under the curve (AUC) of 0.84, which

were greater than those of CRP (AUC = 0.65), the band counts (AUC = 0.74) and the ANC (AUC = 0.75).

Conclusions: Although the band counts and the ANC are also useful for diagnosing acute infection, the neutrophil volume distribution width (NDW) showed superior sensitivity and specificity. In addition, the NDW is a quantitative, more subjective and more accurate parameter generated by Coulter LH750 during automated differentials. We believe that the NDW may be used as an additional indicator for acute bacterial infection.

Kidney

1196 Routine Immunohistochemical Screening of All Renal Transplant Biopsies for Polyomavirus

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Background: Polyoma (BK) virus nephropathy (BKN) is a common and serious complication, occurring in 1% to 8% in renal allograft recipients, and often leads to severe allograft dysfunction and graft loss. The diagnosis of BKN relies on the identification of characteristic viral cytopathic effect in the renal tubular epithelium on light microscopic examination of a graft biopsy. Diagnostic confirmation can be achieved by immunohistochemistry for Simian virus (SV40) and electron microscopy. The incidence of BKN in renal allograft recipients that do not exhibit the diagnostic histological features, and can be diagnosed only by immunohistochemical or molecular techniques is not known.

Design: All non-protocol renal allograft biopsies over a 12 month period were stained for polyomavirus (SV40, clone PAb 280, Oncogene Research Products, Boston, MA) by immunoperoxidase. In addition selected cases from a additional 10 month period were stained for polyomavirus. All light microscopic slides were carefully reviewed by two pathologists independently.

Results: Total number of 302 renal allograft biopsies was identified. Thirteen (4.30%) biopsies with the diagnosis of BKN, from 11 patients were identified. The patients age ranged between 27 and 72 years (mean 46.1 year), and there were 10 males (90.9%), and 1 female (9.09%). The time of allograft renal biopsies ranged from 3 to 53 months post transplantation (mean 18.4 months). On light microscopic examination, 8 (61.5%) biopsies from 7 (63.6%) patients showed classical histological features of polyomavirus cytopathic effect. Five (38.4%) biopsies from 5 (45.4%) patients exhibited a variable degree of reactive changes of the tubular epithelial cells, but no diagnostic viral cytopathic changes. All but one of these 5 patients showed clinical evidence of polyomavirus infection, including positive plasma polymerase chain reaction (PCR) test for BK virus DNA. All 13 renal allograft biopsies were positive for SV40 immunohistochemical stains.

Conclusions: Polyomavirus nephropathy is common complication in allograft renal transplant recipients. Routine screening by immunohistochemical staining for SV40 is recommended for every allograft renal transplant biopsy that performed for renal dysfunction, since it detects a significant number of polyomavirus infection, which is clinically relevant, but otherwise not detected by routine light microscopic examination.

1197 Immunohistochemical Staining for C4d on Formalin-Fixed Paraffin-Embedded Versus Frozen Sections in Renal Allograft Biopsies

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Background: Staining for C4d in renal allograft biopsies, in addition to histologic findings and anti-HLA serology, is considered to be a marker of humoral rejection. Most centers utilize a monoclonal antibody to C4d on frozen sections using either immunofluorescence or immunoperoxidase techniques. Staining paraffin sections can potentially be advantageous due to improved morphology, ease of interpretation, and reducing dependence on requiring frozen tissue. Although the specificity of the polyclonal C4d antibody on paraffin is thought to be high¹, its sensitivity for antibody-mediated rejection is unknown.

Design: We examined 48 non-protocol renal allograft biopsies that were diffusely or focally positive for C4d on frozen sections using a monoclonal antibody (Quidel, Santa Cruz) by immunoperoxidase from two centers. For comparative purposes, an additional 52 consecutive C4d negative biopsies were also studied. Formalin-fixed paraffin sections were stained by immunoperoxidase using a polyclonal antibody against C4d (BIOMEDICA, Salem, NH). Clinical and histological factors were used to help determine whether antibody mediated rejection occurred.

Results: Forty-eight of 100 cases were found to be C4d positive on frozen section using the monoclonal antibody and fifty-two cases were negative. Forty-two of the 48 cases subsequently showed positive staining using the polyclonal antibody on paraffin. Twenty-four of 42 showed strong positive staining (2+ or 3+), 6 showed weak staining (1+) and 12 showed equivocal staining (focal 1+). Of the remaining 6 of 48 cases, 3 showed no paraffin staining for C4d, and 3 were difficult to interpret due to scant tissue. All cases positive on paraffin sections also showed glomerular staining, which often was comparable in intensity to the peritubular capillary staining. Of the 3 cases negative on paraffin sections for C4d but positive on frozen section, all showed either clinical or histologic evidence of antibody mediated rejection. All of the 52 cases that were negative for C4d staining on frozen section were negative on paraffin sections.

Conclusions: Paraffin staining for C4d is not as sensitive for antibody mediated rejection as using the monoclonal antibody against C4d on frozen sections.

1198 De Novo Light Chain Deposition Disease in the Renal Allograft

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Background: Abnormalities which may be seen in renal allograft biopsies include transplant rejection, drug toxicity, infection, damage due to obstruction or vascular compromise, recurrence of primary disease, and least commonly, development of unrelated de novo renal disease. We recently encountered several cases of apparent de novo light chain deposition disease in allograft kidneys. This unusual occurrence prompted further investigation.

Design: Review of our case files from 3-1-01 to 8-31-05 was performed in search of all cases of light chain deposition disease occurring in renal allograft biopsies. Six cases were identified from a total of 1212 allograft biopsies, 2 of which represented recurrent disease occurring in known myeloma patients. Thus, 4 de novo cases were found during this period.

Results: Indications for allograft biopsy in these patients included creatinine elevation in all four, proteinuria in two, and hematuria in one. Primary disease in all four patients was "hypertensive nephropathy." Time post-transplant ranged from 2 to 7.5 years. Light microscopic abnormalities included varying degrees of interstitial fibrosis and tubular atrophy with associated interstitial inflammation. Glomeruli in one case appeared normal by light microscopy. Findings in the remaining cases included mesangial expansion, nodular sclerosis, and segmental sclerosis. Immunofluorescence examination showed tubular basement membrane staining for light chains in all cases. Glomerular staining was seen in the three cases in which glomeruli were included in the immunofluorescence tissue. By electron microscopy, electron dense deposits were seen in the tubular basement membranes in all cases. Glomerular deposits were documented in two of the three cases in which glomeruli were available for assessment. Kappa light chain restriction was seen in three cases with the remaining case showing lambda light chain deposition.

Conclusions: Myeloma patients undergoing transplantation for monoclonal immunoglobulin deposition disease frequently experience recurrence of the disease in the transplanted kidney. A high rate of monoclonal gammopathies has been reported in patients undergoing immunosuppressive therapy following renal transplantation, however, the development of monoclonal immunoglobulin deposition disease is extremely rare according to published literature. The possibility that these cases may reflect changing immunosuppressive regimens should be considered. The actual frequency of occurrence may be underestimated because of lack of routine staining for kappa and lambda light chains in allograft biopsies.

1199 Frequency and Significance of Histopathologic Features of Collapsing Glomerulopathy in End Stage Kidney Disease (ESKD)

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Background: Occasional glomeruli with features of collapsing glomerulopathy (CG) are often present in nephrectomy and biopsy specimens of patients with ESKD of different causes undergoing kidney transplantation. We investigated the frequency of such findings, their morphological and immunohistochemical characteristics, and their clinical background.

Design: Causes of ESKD in 176 cases were determined based on clinical history and histologic findings, and the cases were reviewed for the presence of features of CG. Immunohistochemical stains for synaptopodin, WT-1, Ki-67, cyclinD1, p57, p63, and desmin were performed on randomly selected cases of ESKD, with and without collapsing lesions, using adequate controls.

Results: Features of CG were observed in 33 cases of ESKD (19%). Only two of the 33 cases were HIV-associated (HIVAN); the HIV status was unknown in most other cases, but HIVAN was not clinically suspected in any of them. The profile of causes of ESKD did not differ among cases with and without collapsing lesions. In all studied cases of ESKD, the preserved glomeruli maintained reactivity for cyclinD1 and p57 in podocytes; however, their expression was absent or minimal in glomeruli with features of CG. WT1 expression in glomeruli was variable in all cases regardless of the cause of ESKD, but reduced WT1 expression was observed in collapsed glomeruli. In preserved glomeruli in ESKD cases, synaptopodin was absent or reduced, with greater expression reduction in ESKD cases with features of CG (up to 46%) when compared to ESKD cases without these features (up to 15%). The Ki-67 expression was present in about 23% of glomeruli of ESKD cases with CG features. Marked reactivity for desmin was observed within the inner cell layer of some pseudocrescents.

Conclusions: Histopathologic features of CG are frequently observed in ESKD, causing potential diagnostic difficulties. The immunohistochemical profile of cases of ESKD with CG lesions differs from the profile described for CG in the literature, where profound loss of WT1, synaptopodin, and cyclin D1 was observed. This points to either the non-specific nature of CG lesions in ESKD or to a different (possibly milder) form of CG that can be superimposed on any type of injury seen in ESKD. Prospective studies that would include thorough clinical and laboratory information are needed to more completely define the nature and significance of these lesions.

1200 Necrotizing Granulomatous Tubulointerstitial Nephritis Due to Adenovirus Infection in the Renal Allograft: A Characteristic Morphologic Pattern with Major Clinical Implications

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Background: The characteristic morphologic changes of severe adenovirus infection of the renal allograft are not widely appreciated. Their recognition by the pathologist is vital to diagnosis and successful treatment. An autopsy series of 10 immunocompromised patients described a pattern of necrotizing tubulointerstitial nephritis caused by adenovirus infection. Until recently this pattern has not been observed in living patients. The two cases described here represent the fourth and fifth

such reported cases, and also the first two cases of successful treatment of severe renal allograft adenovirus infection with intravenous ribavirin and IVIG. In both cases the diagnosis was first suggested by the characteristic histomorphologic findings.

Design: Report of two cases and review of the literature

Results: **Case 1** A 46 year old woman presented 22 months after a simultaneous kidney pancreas transplant with fever, malaise, and gross hematuria. Creatinine was increased at 4.8 mg/dl from 1.0 - 1.3 mg/dL baseline. There was no evidence of pancreas graft dysfunction. **Case 2** A 20 year old woman presented with fever, headache, and cough one year after a living related kidney transplant. Creatinine peaked at 8.3 mg/dL from a baseline of 1.3 - 1.5 mg/dL. The renal biopsies showed remarkably similar findings. In each biopsy there was near total involvement by tubulocentric necrotizing granulomatous inflammation with focal destruction of the tubular basement membranes. Some tubular epithelial cells had enlarged nuclei with indistinct amphophilic inclusions. Eosinophils and interstitial hemorrhage were prominent. Glomeruli and blood vessels were spared. Immunohistochemistry for adenovirus was positive within scattered tubular epithelial cells. No "decoy cells" were seen on urine cytology in either case. Both patients had near complete improvement in renal function after combined ribavirin/IVIG therapy.

Conclusions: The pathologist is critical in the diagnosis of adenovirus infection of the renal allograft. Recognition of the characteristic necrotizing granulomatous tubulointerstitial nephritis can result in successful treatment of the infection with near complete restoration of renal function despite the extent, intensity, and destructive nature of the inflammation.

1201 Tubular Basement Membrane Immune Deposits Associated with Polyoma Virus Nephropathy in Renal Allografts

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Background: Tubular basement membrane (TBM) immune deposits are uncommon in renal allografts, and are usually attributed to recurrent or de novo immune complex-mediated glomerulonephritis, such as lupus nephritis or membranous glomerulonephritis. Infection of the renal allograft by polyoma virus is an important cause of graft injury and graft loss, although the pathogenesis of polyoma virus nephropathy (PVN) remains poorly understood. An association between TBM immune deposits and PVN has not been made previously.

Design: We undertook a retrospective study of all renal allograft biopsies reviewed at the University of Washington department of Pathology from January 2000 through September 2005. We identified all cases in which TBM immune deposits were detected by immunofluorescence (IF) and/or electron microscopy (EM).

Results: 3,316 allograft biopsies were reviewed. In biopsies with IF and/or EM, we identified 21 cases with TBM deposits. Four of these cases (20%) showed evidence of immune-complex mediated glomerular disease (2 cases of membranous glomerulonephritis, 1 case of lupus nephritis and 1 case of monoclonal immunoglobulin deposition disease). Fifteen of 21 cases (71%) had polyoma virus infection, diagnosed by the presence of characteristic viral inclusions by light microscopy, and confirmed either by immunohistochemical staining for SV40 antigen or by the identification of characteristic viral particles in ultrastructural studies. Cases with TBM deposits showed no distinct inflammatory pattern by light microscopy, compared to cases of PVN without deposits. TBM deposits were patchy, and were often adjacent to infected tubular cells (characterized by cellular enlargement and/or coating of cells by immunoglobulin). TBM deposits demonstrated a granular pattern by IF and were comprised of various immunoglobulin and complement components. By EM, the deposits were discrete and showed no substructure. In all, 43% of the total number of polyoma virus positive biopsies evaluated by IF and EM during this time period demonstrated TBM deposits.

Conclusions: In renal allograft biopsies without evidence of immune complex mediated glomerular disease, 88% (15 of 17) of the cases with TBM deposits were associated with polyoma virus nephropathy. The frequent localization of TBM deposits immediately adjacent to infected tubular cells provides presumptive evidence of an in situ antibody response, directed either to shed viral antigens or to altered cellular antigens shed from infected cells.

1202 Cyclosporine A and Tacrolimus Has Similar Nephrotoxic Effects in Rats

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Background: Cyclosporine (CsA) and tacrolimus (Tac) were two calcineurin inhibitors. The aim of our study was to compare the cytokine expression in a rat model of chronic CsA and Tac nephrotoxicity in rats.

Design: Twenty-four male Wistar rats were divided into three groups of eight: Group 1(G1): 8 healthy controls; group 2(G2) : 8 treated with Cs A (15mg/kg/day intraperitoneally); group 3(G3):8 treated with Tac (1 mg/kg/day intraperitoneally) both for 8 weeks. Serum drug levels, creatinine and creatinine clearance were performed at the end of the study period. Renal tissue were assessed for light microscopic findings of Tac and CsA toxicity and TGF-β, VEGF, BMP-7 expression were semiquantitatively scored after immunohistochemical staining.

Results: Mean CsA level was 1298±21ng/ml in group 2, mean Tac level was 13±9.52ng/ml respectively. At the end of the study period creatinine clearance was 1.64±0.08ml/min in group 1, 0.35±0.03ml/min in group 2 and 1.02±0.36ml/min group 3(G1vsG2 and G3 <0.001).Morphological changes including interstitial fibrosis and hyalen arteriolar hyalinosis were significantly increased in calcineurin treated rats compared to control group. Within the calcineurin inhibitors, interstitial fibrosis and hyalen arteriolar hyalinosis were similar in G2 and G3. Tubulointerstitial immunoeexpression of TGF-β and VEGF was significantly increased in G2 and G3 compared to G1 whereas BMP-7 expression was significantly decreased in G2 vs G3 but the difference failed to reach statistical significance between G1 and G3. TGF-β, VEGF and BMP-7 expression were similar in G2 and G3.

Conclusions: In conclusion although GFR seemed to be better preserved in Tac treated group compared to CsA group both tacrolimus and cyclosporine had same morphologic and immunohistochemical findings in the rat model of calcineurin nephrotoxicity.

1203 Effects of Long-Term Administration of High-Dose Recombinant Human Antithrombin in Immunosuppressed Primate Recipients of Porcine Xenografts

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Background: Fibrin deposition is central to the acute humoral rejection process occurring in the presence of consumptive coagulopathy when pig organs are transplanted into primates

Design: To assess whether strategies aimed at preventing fibrin formation may extend xenograft survival, we administered high daily doses of recombinant human antithrombin (rhAT) (500 U/kg twice daily) to obtain both anticoagulant and anti-inflammatory effects in immunosuppressed primate recipients of porcine kidneys.

Results: Some degree of consumptive coagulopathy developed in both rhAT-treated (n=3) and untreated (n=3) primates. No major differences in the coagulation parameters analyzed were observed between the 2 groups. Similarly, no difference in survival was seen between rhAT-treated (20.6 ± 4 days; range: 15-23 days) and untreated animals (17.3 ± 11.6 days; range: 7-30 days), although the rhAT-treated primates had a higher bleeding tendency. Despite the high daily dose of rhAT, considerable fibrin deposition was observed in the graft as early as 2 weeks after transplantation.

Conclusions: These results suggest that a high daily dose of rhAT fails to influence survival or prevent fibrin formation and deposition in the graft in our pig-to-primate model. However, the potential role of rhAT administered in combination with heparin or other clotting inhibitor concentrates in this model remains to be determined.

1204 Influence of Pglycoprotein in Cyclosporin (CSA) Toxicity: Conventional and Computerized Image Analysis

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Background: CSA toxicity has unic histologic findings and its chronic ischemic nephrotoxicity is an obstacle of its use in allograft transplantation. Experimental murine rodent models are complex due to difficulty in lesion reproducibility, requiring high doses distant from those used in clinical practice, hampering the model interpretation. The aim is to evaluate tubulointerstitial fibrosis by computerized image analysis to demonstrate an increase of it in a model of CSA chronic toxicity of P Glycoprotein knockout (KO) mice (mdr1a/b).

Design: 64 male FVB, wild type (WT) and Pglycoprotein KO mice underwent hypodermic regime and subcutaneous administration of CSA or vehicle (olive oil) for 42 days in groups of six animals with 3 different regimes: 1) CSA 75 mg/kg 2) CSA 35 mg/kg 3) Vehicle. After treatment serum creatinine and BUN were established and conventional microscopic evaluation of cortical tubulointerstitial fibrosis was performed (% of lesionated fields on 50 HPF (400x), Olympus BX51, field area: 0.85 mm², trichromic staining). The groups of CSA 35 were also analyzed quantitatively with automated scanning microscope and digital analysis (30% of cortex was evaluated, unit of interstitial space lesion: Kpixel/HPF, staining: sirius red (Ariol Scan 2.1, Ariol SL-50 Applied Imaging Corp, Olympus 2005). Statistical analysis consisted of non-parametric Mann-Whitney Test.

Results: No differences in renal function were observed between KO and WT exposed at any dose (CSA 75 WT vs KO BUN: 51 ± 10.8 vs 90 ± 39.5 mg/dl (p: 0.09). CSA 35 WT vs KO BUN: 45 ± 6.5 mg/dl vs 60.2 ± 10.9 (p: 0.07). By conventional microscope KO CSA 75 group showed an increased lesion compared to WT (48.9 ± 15.7 % vs 7.5 ± 3.5 %) (p:0.003). However, no difference was observed in CSA 35 treatment between KO and WT (5.3 ± 5 vs 1.7 ± 1.9 %) (p: 0.2). By digital analysis KO CSA 35 group showed a significant increased lesion compared to WT (24.6 ± 13.2 vs 0.6 ± 0.7 KPixel HPF) (p: 0.01). No differences were observed between KO and WT treated with vehicle.

Conclusions: The absence of expression of P Glycoprotein inflicted an increase in the lesions of CSA toxicity in both groups of 75 and 35 mg/kg. In the group of 35 mg/kg (dose similar to clinical treatment) the lesions were just proven using digital quantification. The use of quantitative histopathologic analytical tools is useful in the evaluation of experimental models of nephrotoxicity by CSA.

1205 A Clinicopathologic Study of Renal Manifestations in Patients after Bone Marrow Transplantation

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Background: Bone marrow transplantation (BMT) is a common treatment option for a variety of hematopoietic malignancies. Due to the use of radiation and/or chemotherapeutic agents, renal dysfunction often ensues. Many pharmacologic agents have been linked with thrombotic microangiopathy (TMA), and an association between membranous glomerulonephritis (MGN) and graft-versus-host disease (GVHD) has been reported in the setting of BMT. We conducted a single institution study of BMT patients who developed renal dysfunction to document the wide histologic spectrum of renal manifestations.

Design: We reviewed the pathology files at the University of Washington Medical Center (Seattle, WA) from 1999-2004 and identified 11 patients with both a renal biopsy and a history of BMT. We correlated the histologic findings with relevant clinical information.

Results:

Case	Age	Sex	Orig Dz	Clinical Sx	GVHD	Renal biopsy
1	56	F	ALL	TTP-like sxs	No	TMA, CIT, ATN
2	65	M	Amyloidosis	Proteinuria	No	Amyloidosis
3	27	M	AML	Proteinuria	No	MGN, ATN
4	60	M	MM	Proteinuria	Yes	MCD, CIT, TBM depts, obliterative arteriopathy
5	40	M	AML	Proteinuria	Yes	MCD, ATN
6	42	M	NHL	Azotemia	No	BK virus neph, TBM depts
7	38	M	AML	ARF	Yes	ATN
8	55	M	MM	Renal insufficiency	Yes	FSGS, "tip" variant
9	63	M	MM	Renal insufficiency	No	Cast neph, LCDD
10	60	M	CLL	Proteinuria	No	MGN with atypical features
11	72	M	Amyloidosis	Proteinuria	No	Amyloidosis

Abbreviations: Sx-symptom; Orig Dz-original disease; ALL-acute lymphocytic leukemia; AML-acute myeloid leukemia; NHL-non-Hodgkin lymphoma; MM-multiple myeloma; CLL-chronic lymphocytic leukemia; TTP-thrombotic thrombocytopenic purpura; ARF-acute renal failure; CIT-calcineurin inhibitor toxicity; ATN-acute tubular necrosis; MCD-minimal change disease; TBM depts-tubular basement membrane immune deposits; FSGS-focal segmental glomerulosclerosis; neph-nephropathy; LCDD-light chain deposition disease

The renal manifestations presented between 7 months to 5 years after BMT. 5 patients had severe proteinuria (15 to 26 grams/day).

Conclusions: Due to the numerous treatment modalities and potential toxic agents during BMT, it was difficult to attribute the subsequent renal dysfunction to a specific factor. Three cases represented recurrent manifestations of the original diseases. The remaining renal biopsies revealed a wide spectrum of one or more injuries to the different compartments of the kidney. MGN and MCD were the most common causes of severe proteinuria, of which only the MCD cases in our series were associated with clinical evidence of GVHD.

1206 Mesangial Cell Versus Monocyte Interposition in Membranoproliferative Patterned Glomerulonephritides

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Background: In recent years, monocytes have been promoted over mesangial cells as the interposing cells in membranoproliferative-patterned glomerulonephritides. This has especially been the case regarding Hepatitis C related membranoproliferative glomerulonephritis (MPGN).

Design: To test this hypothesis, consecutive sections of kidney biopsies with membranoproliferative patterns (i.e. MPGN I or III, Lupus class III or IV) were immunohistochemically stained with smooth muscle actin (SMA) to identify mesangial cells and CD68 to identify monocytes. All slides were subsequently stained with PAS in order to pinpoint the exact position of interposing cells relative to the glomerular basement membrane (GBM) and to estimate the percentage of doubly contoured glomerular capillary loops per glomerulus, averaged per case. This study included 7 cases of MPGN and 11 cases of proliferative lupus nephritis. The Student's T-test was used to determine whether a significant difference exists between mean percentage of glomerular capillary loop mesangial cell interposition and mean percentage of glomerular capillary loop monocyte interposition amongst all cases together. Fisher's exact test was used to determine whether mesangial cell versus monocyte interposition was significantly correlated with proliferative lupus nephritis versus MPGN.

Results: 1) Mean percentage of glomerular capillary loops with interposing mesangial cells was significantly greater than mean percentage of glomerular capillary loops with interposing monocytes (36.9 ± 28.2 versus 7.88 ± 13.5 , $p=0.0004$), regardless of whether the MPGN-like pattern was due to true MPGN or proliferative lupus nephritis. Interposing mesangial cells were also identified in numerous glomerular capillary loops lacking doubly contoured basement membranes. 2) Although the highest percentage of capillary loops with interposing monocytes belonged to a case of hepatitis C-related MPGN, there was no significant correlation between interposing cell type and MPGN versus proliferative lupus nephritis ($p=0.267$). 3) Although not statistically calculated, cases with the highest percentages of monocyte interposition contained exceedingly higher numbers of endocapillary intraluminal monocytes.

Conclusions: Despite reported observations of many interposing and endocapillary intraluminal monocytes in various MPGN-patterned glomerulonephritides, mesangial cells constitute the significant majority of interposing cells in all such cases.

1207 Distinctive Ultrastructural Features of Chronic Allograft Glomerulopathy: New Formation of Circumferential Glomerular Basement Membrane

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Background: Chronic allograft glomerulopathy (CAG), defined as duplication of the GBM in the absence of deposits, has a variety of causes. This study was undertaken to ascertain features that might distinguish two of the leading causes, chronic allograft rejection and thrombotic microangiopathy (TMA).

Design: Electron microscopic studies of allografts with CAG (n=10) were compared with those of native kidneys with GBM duplication due to other causes, TMA (n=14), lupus nephritis (n=27) and membranoproliferative glomerulonephritis (MPGN), type I (n=3).

Results: Duplication of the peripheral GBM was present in all CAG and TMA cases. However, there was circumferential lamination (≥ 2 layers) of the basement membrane, including neof ormation of the GBM between endothelium and mesangium in 70% of the CAG cases but none in the TMA cases ($p<0.001$). Peritubular capillary (PTC) circumferential lamination (≥ 2 layers) was found in 80% of the CAG cases and none in the TMA cases ($p<0.001$). Four of the CAG cases had concomitant C4d deposition in

the PTC. One additional case had a prior biopsy with C4d. Circumferential GBM lamination was not found in 27 cases of lupus nephritis or 3 cases of MPGN.

	Basement Membrane Ultrastructure			PTC Lamination
	Peripheral	Neof ormation of GBM Circumferential (1 layer)	Circumferential (>=2 layers)	
Transplant				
CAG	10/10 (100%)	8/10 (80%)*	7/10 (70%)*	8/10 (80%)*
Native Kidneys				
TMA	14/14 (100%)	1/14 (7%)	0/14 (0%)	0/9 (0%)
LN	27/27 (100%)	0/27 (0%)	0/27 (0%)	0/3 (0%)
MPGN	3/3 (100%)	0/3 (0%)	0/3 (0%)	0/3 (0%)

* - p<0.001 vs. TMA

Conclusions: The presence of circumferential lamination of the GBM is characteristic of CAG and may be a useful diagnostic finding that supports a diagnosis of rejection vs. TMA. Documentation of C4d deposition at the time of, or before, biopsy is common but not universal in CAG (50%), suggesting that some of these lesions may be either T cell mediated or the sequelae of previous undocumented episodes of humoral rejection.

1208 IgG4 Immune-Complex Tubulointerstitial Nephritis Associated with Autoimmune Pancreatitis

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Background: Autoimmune pancreatitis is a mass-forming chronic fibroinflammatory condition characterized by predominant IgG4-positive plasma cells. Two cases from Japan have been reported associated with interstitial nephritis. We describe four additional renal cases and characterize the immunological features.

Design: Renal biopsy or nephrectomy cases showing tubulointerstitial nephritis were identified from patients with autoimmune pancreatitis. Light, immunofluorescent (IF), immunohistochemical (IHC), and electron microscopic features were characterized.

Results: Four cases of tubulointerstitial nephritis associated with autoimmune pancreatitis were identified. The specimens consisted of 3 biopsies and one nephrectomy. The average patient age was 69 yrs (range 57-78); all had histologic and/or clinical and radiographic evidence of autoimmune pancreatitis. The clinical impression in three patients was a renal mass or vasculitis. The fourth patient underwent biopsy for renal insufficiency. Histologic preparations revealed a dense interstitial lymphoplasmacytic infiltrate. Eosinophils were often numerous. Tubulitis and tubular injury were present, along with tubular atrophy with focally thickened tubular basement membranes (TBM); deposits were evident in the TBM in trichrome stains. Two cases showed striking expansive interstitial fibrosis with tubular destruction, while two had a cellular, inflammatory pattern. "Venulitis" as described in autoimmune pancreatitis was observed in two cases. The nephrectomy demonstrated a mass-like nodular pattern of inflammation with normal renal tissue elsewhere. Glomeruli were entirely normal or showed mild mesangial hypercellularity. Many plasma cells were positive for IgG4 by IHC. TBM granular IgG deposits, predominantly of the IgG4 subclass, were detected in 3 of 4 cases by either IF or IHC. Other TBM findings by IF included granular C3 deposition. By electron microscopy, corresponding amorphous electron-dense deposits were present in the TBM in 3 of 4 cases. Glomeruli showed no deposits.

Conclusions: We describe four cases of a new entity, tubulointerstitial nephritis associated with autoimmune pancreatitis, characterized by a mass-like lesion consisting of a dense lymphoplasmacytic infiltrate with eosinophils and prominent IgG4-positive plasma cells and immune complex deposits in the TBM. This type of tubulointerstitial nephritis may be part of a systemic IgG4-related disease, which we term "multi-organ autoimmune IgG4 immune-complex disease" (MAID).

1209 Podocyte Gene Expression Profiling in an In-Vitro Model of Diabetic Nephropathy

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Background: Podocyte loss has been suggested to play an important role in the pathogenesis of diabetic nephropathy but little is known about the molecular changes that support podocyte structure and function. To gain an insight into the molecular perturbations that may explain podocyte injury in diabetes we performed global gene transcript profiles in podocytes exposed to high glucose concentration in vitro.

Design: Mouse podocytes conditionally immortalized were exposed to media containing normal Glucose concentration (NG) as a control 5.5 mmol/L D-glucose or High Glucose (HG) 25 mmol/L D-glucose, for 1 and 2 weeks. mRNA was isolated from treated and control cells (in duplicate) at 1 week and 2 weeks post treatment and hybridized to mouse GeneChips (Affymetrix MOE430+2) that contain approximately 45,000 transcripts. We used dChip software to analyze the data. A cut-off of <3 and >3 fold change was considered significant (P < 0.05).

Results: A filtered set of 16,000 genes had varied expression. These include many known podocyte genes such as synaptopodin and podocin. We found 39 new transcripts significantly down-regulated, 12 in HG at 1 week and 27 in 2 weeks. An equal number (39) of transcripts were upregulated, of which 14 in 1week and 25 in 2 weeks HG treated podocytes. Altered genes included extracellular matrix modulators, cell cycle regulators, extracellular transduction signals and membrane transport proteins. Novel genes that were altered in both of the treated groups included neutrophil gelatinase-associated lipocalin (LCN2) which was decreased by 3.2 fold at 1 week and by 7.2 fold at 2 weeks, Endothelial lipase (EL) - increased by 3.57 fold at 1 week and 3.88 fold at 2 weeks and UDP-glucuronosyltransferase 8 (Ugt8) which was increased by 3.9 fold at 1 week and 4.98 fold at 2 weeks. LCN2 has been shown to be important in preventing tubular epithelial cell apoptosis in ischemic renal injury and EL is known to protect cells from antioxidant stress and promotes monocyte adhesion suggesting a possible role of these molecules in diabetic podocyte injury. Ugt8 is known to have

a role in neural myelination that may explain coexistence of nephropathy with neuropathy in a subset of patients with diabetes.

Conclusions: LCN2, EL and Ugt8 are consistently altered in podocytes exposed to HG in timed biological experiments and suggest novel mechanisms mediating podocyte injury in diabetic nephropathy.

1210 Dystroglycan in Diagnosis of FSGS

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Background: α - and β -dystroglycan (DG), which links the actin cytoskeleton of the podocyte to the GBM has been described as maintained in FSGS, but decreased in minimal change disease (MCD). Fibrosis has been linked to increased tubulointerstitial expression of fibroblast-specific protein-1 (FSP-1) and epithelial-mesenchymal transition (EMT). We investigated whether podocyte differentiation (WT-1), DG and FSP-1 varied in different subclasses and could help in diagnosis of FSGS.

Design: Renal biopsies with diagnosis of primary FSGS, nos variant (NOS, n=11), tip lesion variant (TIP, n=8), collapsing lesion variant of FSGS (COLL, n=5), vs secondary FSGS (SEC, n=8), or cases without segmental sclerotic lesions where a diagnosis of MCD vs FSGS could not be established (undefined, UNDEF, n=10) were studied and compared to normal (n=4) by immunohistochemistry. Staining was scored on a 0-3+ scale.

Results: WT-1 was markedly decreased in NOS vs normal (1.7 \pm 0.3 vs 3.0 \pm 0, p=0.035) and tended to correlate with the extent of sclerosis. β - and α -DG were maintained in most of both primary and secondary FSGS cases. In contrast, α -DG was significantly decreased in UNDEF (p = 0.02 vs normal), supporting a diagnosis of MCD. Further, follow-up showed remission or decreased proteinuria in 4 of these UNDEF cases in response to therapy. Interstitial FSP-1 was increased in COLL (0.7 \pm 0.3 vs normal 0 \pm 0), but was only rarely found in tubules or podocytes in any FSGS cases.

Conclusions: We conclude that increased FSP-1 may be a marker of the aggressive course of collapsing FSGS. Further, DG staining may be a useful adjunct to assist in distinction of FSGS vs MCD in biopsies without defining lesions.

1211 Glomerular Galectin 3 (Gal) Expression in Lupus Nephritis. Pathologic and Biologic Implications

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Background: Gal participates in the regulation of cell proliferation, differentiation and apoptosis. Preliminary data indicate a role for Gal in renal diseases, including diabetic nephropathy. We evaluated glomerular expression of Gal and focused on its biologic significance in lupus nephritis.

Design: Gal expression was studied in 85 specimens: normal kidney (10), proliferative glomerulonephritis (GN, 13), nonproliferative GN (17), and lupus nephritis (12 Class II, 13 Class III, 12 Class IV, and 8 Class V). Lupus nephritis cases were also stained for macrophages and lymphocytes. Gal expression was quantified as number of positive cells per glomerulus and was correlated with the activity index, chronicity, interstitial injury and sclerotic glomeruli for lupus nephritis as a group and for each lupus nephritis class.

Results: Gal was not found in normal glomeruli. Gal expression was increased in proliferative GN compared with nonproliferative GN (0.14 vs 1.6). Gal expression was highest in lupus nephritis (1.8) and correlated with lupus class (0.8, 1.7, 3.5, and 0.9 for class II, III, IV, V, respectively). Gal+ glomerular cells included both native glomerular cells and inflammatory cells. With lupus nephritis, there was a strong correlation between Gal expression and the activity index (p=0.00014). This correlation was noted for each lupus nephritis class. Gal expression was strongly correlated with severity of glomerulosclerosis and interstitial injury for Class IV lupus nephritis (p < 0.0013).

Conclusions: Gal appears to play a role in the pathogenesis of proliferative GN, especially in lupus nephritis. Gal is not expressed in nonproliferative GN. With lupus nephritis, Gal expression may serve as a novel marker for disease activity and perhaps, carry prognostic significance.

1212 Granulomatous Pyelonephritis Associated with Urinary Obstruction. A Comprehensive Clinicopathologic Study

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Background: Urinary obstruction is rarely associated with a distinct granulomatous inflammation that involves the pyelocaliceal system and closely simulates infectious conditions, including tuberculosis. Its clinicopathologic features, however, have not been adequately studied, since there are only seven isolated reported cases.

Design: In a comprehensive study of obstructive uropathy, which included at least 112 cases, we found five cases of granulomatous chronic pyelonephritis (GPN). The features of these cases were detailed and compared with the previously reported cases.

Results: The patients included two men and three women between the ages of 38-81 years. All had unilateral urinary obstruction due to lithiasis (3) or ureteral stenosis (2), treated currently or in the past with stent. Three had end-stage renal disease or chronic renal insufficiency. The pyelocaliceal system showed frank hydronephrosis (1) or partial dilatation (4) and contained cheesy and gritty material in its lumen. Each case showed severe granulomatous inflammation, which was limited to the pelvic wall and closely associated with calcified debris, necrotic inflammatory cells and material consistent with Tamm-Horsfall protein. The kidney showed chronic tubulointerstitial nephritis but without granuloma. Cultures of urine, blood, and the renal pelvic content, and

special stains did not show fungi or mycobacteria in any case. Many of these features were also observed in previously reported cases.

Conclusions: GPN is a rare but distinct clinicopathologic entity, in which severe granulomatous inflammation limited to the renal pelvis is uniformly associated with urinary obstruction and pyelocaliceal dilatation and may develop in response to accumulated calcified material in the renal pelvis. Awareness of this entity affords its straightforward and accurate diagnosis with significant treatment and prognostic implications.

1213 Follow up of 31 Cases of Focal Segmental Glomerulosclerosis

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Background: Focal segmental glomerulosclerosis (FSGS) is a common glomerular disease that frequently progresses to end stage renal disease (ESRD). This study evaluates clinical and laboratory factors associated with disease progression.

Design: Patients with biopsy diagnosed FSGS from 1990-1999 were retrospectively identified. Biopsy material was reviewed and clinical information was collected on age, sex, race, serum creatinine, cholesterol, 24-hour proteinuria at biopsy, and development of ESRD (dialysis or transplantation). The biopsies were sub-classified based on a recent classification proposed by D'Agati and colleagues.

Results:

Demographics		Therapy (patients)	
Age	40.2 (±16)	ACE Inhibitors	16
Sex		Steroids	9
Male	21 (68%)	Cyclosporin	8
Female	10 (32%)	Combination of 2 or 3 drugs	9
Race		No treatment	11
African American	19 (61%)		
Caucasian	12 (39%)		

Overall, 31 cases were identified. The mean follow-up time was 192 mths (range 5 to 368 mths). Twelve patients (38.7%) developed ESRD with the average time from biopsy to the development of ESRD being 7.8 yrs (range 1-12yrs). The biopsies were sub-classified according to the proposed scheme as follows: 18(58%) not otherwise specified (NOS), 12(39%) perihilar, 1(3%) collapsing variant. No cellular or tip variants were identified. Mean times for patients with perihilar FSGS and FSGS, NOS to develop ESRD was 249(±18) mths and 172(±103) respectively. Four patients (33%) with perihilar FSGS and 7(38%) with FSGS, NOS developed ESRD (p=NS). The one patient with collapsing FSGS developed ESRD 8 months after diagnosis. ESRD was more common in females than in males (60% vs. 33%, p=0.08). The mean level of serum creatinine, at the time of biopsy, for patients who developed ESRD was 2.55 ±1.68 mg/dl compared to 1.59 ±1.19 mg/dl for those who did not (p=0.09). The mean 24 hr urine protein at the time of biopsy for patients who developed ESRD was 7.72 ±4.21 mg compared to 5.46 ±5.22 mg for those who did not (p=NS). Univariate analysis of each therapy suggested that use of ACEI was not associated with a reduced risk of developing ESRD (Relative Risk(RR) =1.56, p=NS). Use of steroids and cyclosporin were associated with a five-fold (RR=5.3, p=0.04) and eight-fold (RR=8.5, p=0.01) reduced risk respectively.

Conclusions: 1. Treatment with steroids or cyclosporin is associated with a reduced risk of developing ESRD. 2. Patients who developed ESRD had higher initial serum creatinine than those who did not, approaching statistical significance. 3. Females developed ESRD at a greater rate than males, approaching statistical significance.

1214 Comparison of CD20+ Cell Rich and Plasma Cell Rich Infiltrates with Poor Graft Outcome in Acute Cellular Rejection of Renal Allograft

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Background: Biopsy proven acute rejection (BPAR) is a negative prognostic factor for long-term renal allograft survival. Many histopathological characteristics affect response to anti-rejection therapy and graft outcome. Our aim is to compare CD20+ B lymphocyte rich infiltrates with plasma cell rich infiltrates in BPAR with special attention to graft survival or loss.

Design: We retrospectively reviewed renal allograft rejections from year 1998-2002. Of the 385 renal transplants, 59 had BPAR. Tissue was available in 32 patients (41 biopsies) for reevaluation for Banff classification, quantifying the amount of plasma cells and lymphocytes, and for immunohistochemical staining for C4d, CD4, CD8, CD20 and VS38. Information was collected about age, sex, race, immediate vs. delayed graft function, acute rejection and treatment, need for hemodialysis and graft survival. Plasma cell rich infiltrate was defined as plasma cells more than 30% of interstitial infiltrate in the biopsy. CD20+ rich infiltrate was defined as CD20+ cells more than 12.5% of interstitial area of the biopsy (minimal 2.5 times the value for CD20+ poor infiltrate), and CD20+ poor if CD20+ cells were less than 5% (excluding cases with plasma cell rich infiltrate).

Results: Sixty-six percent of patients were African-American, 40% deceased donor, 51% living-related, 8.5% living unrelated transplants and 89% were first transplant. There were 13 Banff type 1a (32%); 12 type 1b (29%); 7 type 2a (17%) and 9 type 2b (22%) rejections in the biopsies. C4d staining was positive in biopsies of only 3 (9%) patients. Plasma cell rich infiltrate was identified in 7 patients (22%). At a mean follow up of 11±10 months after the rejection episode, five of these 7 patients (71%) lost their grafts as compared to 12% (3/25) graft loss in patients with plasma cell poor infiltrate (<30%) (p=0.0048). CD20+ rich infiltrate was identified in 12 patients (38%). At a mean follow up of 11±10 months after the rejection episode, two of these 12 patients (17%) lost their grafts as compared to 9% (1/11) graft loss in patients with CD20+ cell poor infiltrate (p=0.5342). These 2 CD20+ cell rich cases also had plasma cell rich infiltration.

Conclusions: Plasma cell rich infiltrate was seen in 22% BPAR and was associated with poor graft survival. CD20+ cell rich infiltrate was seen in 38% of BPAR and appeared not associated with poor graft survival, although the sample number is small.

1215 Expression of eNOS Is Attenuated in the Renal Arterioles and Small Arteries in Patients with Malignant Hypertension

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Background: The importance of the endothelial isoform of nitric oxide synthase (eNOS) has been well established. Endothelium-derived nitric oxide (NO) was essential for vascular homeostasis. Studies of animal models suggest that low level of NO may be essential for normal renal function and cell protection, while high local concentrations of nitric oxide may result in initiation and progression of renal disease. However, little is known regarding eNOS expression and potential modulators in human kidneys. The purpose of this study was to compare patterns of eNOS expression in normal human kidneys, and in patients with hypertensive disease.

Design: Normal kidney tissue sections away from tumor obtained from nephrectomy specimens (5) were used as controls. Kidney biopsies from patients with long-standing benign hypertension (5) and kidney biopsies from patients with malignant hypertension (6) were studied. Routine histochemical stains for kidney biopsies were performed and evaluated. Immunohistochemical stain for eNOS was performed using polyclonal antibodies directed against eNOS.

Results: In the normal kidney, strong staining for eNOS was identified in endothelial cells in arterioles, small arteries and peritubular capillaries with similar intensity. In glomeruli, only weak staining was seen in scattered endothelial cells. In patients with long-staining benign hypertension, the pattern and intensity of eNOS appeared similar to that in normal kidneys. However, in patients with malignant hypertension, the expression of eNOS was significantly attenuated in arterioles and small arteries. The intensity of eNOS expression in glomeruli, medium sized arteries and peritubular capillaries was unchanged.

Conclusions: eNOS is widely distributed in the endothelial cells in the human kidneys. The expression of eNOS is higher in arterioles, small arteries and peritubular capillaries compared than in glomeruli. The intensity and the patterns of vascular eNOS expression is similar in patients with benign hypertension. Nevertheless, eNOS expression is significantly attenuated in patients with malignant hypertension in parts of the renal vasculature. These findings suggest that attenuated expression of eNOS in arterioles and small arteries is a result of the severe disturbance in vascular homeostasis that occurs in parts of the renal vasculature.

1216 Lesions Associated with Plasma Cell Dyscrasias in Renal Biopsies: A 10 Year Retrospective Study

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Background: The files of a referral renal biopsy service were reviewed to analyze renal lesions associated with plasma cell dyscrasias (PCD) detected in the last 10 years. A total of 4,675 renal biopsies were examined during the time period selected and 93 biopsies revealed evidenced of plasma cell dyscrasia-associated lesions. The renal biopsy specimens were received from seven states and more than 40 nephrology practices. The patients with PCD-associated renal lesions ranged from 32 to 88 years of age and about half of the patients did not have a clinically recognized plasma cell dyscrasia prior to the renal biopsy.

Design: The cases were reviewed noting demographic data and clinical presentation, as well as final pathologic diagnosis. Special attention was given to identify cases with subtle pathologic alterations and combined pathologic patterns.

Results: Renal lesions associated with PCD were seen in approximately 2% of all biopsies. Glomerular pathology including light and heavy chain-associated amyloidosis (AL/AH-Am) and light and heavy chain deposition disease (L/HcDD) comprised 60% of the lesions identified and the remainder 40% of the cases included myeloma cast nephropathy (MCN), inflammatory tubular interstitial nephritis (TIN) and acute tubulopathy-AT- (including light chain- associated Fanconi syndrome). The most common lesion identified in this series was AL-amyloidosis (43% of all the cases), followed by myeloma cast nephropathy (21% of the cases). Combined lesions i.e. LCDD and AL-Am and LCDD and MCN were only seen in approximately 2% of all patients. Male to female ratio was essentially equal in most entities with the exception that the two patients with HCDD were male, female patients were twice as common in the interstitial nephritis group (3 males: 6 females), and a slight preponderance of male patients in AL-amyloidosis (22 males: 18 females). The mean patients' age for the various groups was as follows: 72.6 for TIN, 66.3 for MCN, 66 for AT, 60.2 for LCDD, 60.1 for AL-Am and 32.5 for HCDD.

Conclusions: Approximately 2% of all renal biopsies revealed lesions associated with PCD. This study highlights a high incidence of AL-amyloidosis in these patients. Unexpectedly, this lesion was more prevalent than MCN in this series. The mean patients' age for the various groups was above 60 years of age with the exception of HCDD.

1217 Polyoma Virus Antigen-Associated Tubular Basement Membrane Immune Complex Deposition

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Background: Polyoma virus infection is a well known cause of renal dysfunction in kidney transplant recipients. However, there have been no reports of immune complex lesions associated with this virus in humans. We identified 3 renal allograft recipients with polyoma virus infection biopsied for elevated serum creatinine levels. All had polyoma virus identified in tubular epithelium as well as immune complex deposition in tubular basement membranes and were assessed for this study.

Design: Patient charts were reviewed for clinical information. Biopsies were evaluated in the standard fashion. Polyoma virus antigen was identified using indirect immunofluorescence with SV40 antibody. Controls included biopsies with known polyoma virus infection without immune complexes, lupus with tubulo-interstitial deposits, IgA nephropathy and acute transplant rejection.

Results: All patients were female and were 31, 48 and 61 years of age. Transplants were in place for 1-3 years with serum creatinine of 1.7 - 3.9 mg/dl at the time of biopsy.

Immunosuppression consisted of prograf and steroids in all, and celcept in two. Primary renal diseases included diabetic nephropathy, polycystic kidney disease and chronic glomerulonephritis. Viral titers available for 2 patients were markedly elevated in excess of 1 million viral DNA copies, reduced following treatment, then elevated again at the time of biopsy. Biopsy in all demonstrated polyoma virus infection with severe tubulo-interstitial inflammation and a patchy distribution. There was no acute rejection. Tubular basement membranes contained immune complex electron dense deposits staining for IgG and C3 with positive staining of deposits for SV40 polyoma antigen. No glomerular immune complexes were present. All patients were treated with Arava and 1 additionally with cidofovir. Two patients progressed to chronic renal failure at 5 and 10 months after diagnostic biopsy. The patient with initial creatinine of 1.7 had preserved renal function at 10 months after biopsy.

Conclusions: This is the first report in humans of immune complex deposits containing polyoma virus antigen. This occurred in association with intermittent high viral titers and only in tubular basement membranes, possibly indicating in situ immune complex formation with viral antigen and circulating viral antibody; similar findings have been noted with adenovirus infection. The clinical significance is uncertain but may portend a worse prognosis.

1218 Aldose Reductase Regulates TGF β 1-Induced Production of Fibronectin and Type IV Collagen in Cultured Rat Mesangial Cells

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Background: To study the effects of aldose reductase (AR) on TGF β 1-induced production of fibronectin and type IV collagen in cultured rat mesangial cells (MsC). **Design:** Eukaryotic expressing vector, pcDNA3-AR was constructed using digestion, ligation based on prokaryotic expressing plasmid pET-15b-AR. Lipofect AMINE was used for stable transfection and G418 was used for selecting positive clones. Aldose reductase inhibitors (ARI) sorbinil and zopolrestat were added for suppressing the activity of AR. The production of fibronectin and type IV collagen and the activation of Smads and MAPK signal transduction pathway including ERK, JNK, p38 was analyzed by Western blot and the transcription factor AP-1 activity was examined by EMSA.

Results: Normal MsC showed increased expression of fibronectin and type IV collagen with stimulation of TGF β 1. Compared with the normal MsC, the MsC pre-incubated with ARIs showed reduced level of fibronectin and type IV collagen expression ($P < 0.05$), while increased expression was seen in the AR-transfected MsC ($P < 0.05$). In terms of the products within the signal transduction pathway, normal MsC showed activation of ERK, JNK, and p38 under the stimulation of TGF β 1, and the activation of JNK and p38 was inhibited in the MsC pre-incubated with ARIs. However, the activation of JNK was enhanced in the AR-transfected MsC. In addition, the activation of ERK and Smad2 showed no obvious alterations in the ARI pre-incubated or AR-transfected MsC. Enhanced AP-1 activity was seen in normal MsC and more activated activity was observed in AR-transfected MsC when TGF β 1 was added, while the activity were inhibited in the MsC pre-incubated with ARIs.

Conclusions: AR may regulate the expression of fibronectin and type IV collagen with the stimulation of TGF β 1 in rat mesangial cells, which may be related with the activation of JNK-MAPK and p38-MAPK signaling pathway and transcription factor AP-1. These data suggest that AR may play a role in pathogenesis of glomerulosclerosis.

1219 Expanding the Spectrum of Proximal Tubulopathy Associated with Plasma Cell Dyscrasia; Appraisal of Five Patients from a Single Institution

U Kapur, R Fresco, MM Picken. Loyola University Medical Center, Maywood, IL. **Background:** Plasma cell dyscrasia (PCD) is associated with a wide array of renal pathologies. While light chain (LC) cast nephropathy involves predominantly distal tubules, proximal tubules only rarely show diagnostic pathology such as intracytoplasmic crystals. We sought to look for additional features which may be helpful in the diagnosis of proximal tubulopathy (LCPT) associated with an underlying PCD.

Design: We reviewed biopsies from patients who, either at presentation or subsequently, were diagnosed with an underlying PCD. The LM, IF and EM features were studied and correlated with clinical and follow-up data. There were 5 patients with LCPT: 3 females and 2 males.

Results: One patient had a prior diagnosis of multiple myeloma and mild LC proteinuria. Two patients had renal failure and 2 had proteinuria alone. On LM, only 2 patients had focal rhomboid crystals in proximal tubular epithelium, while in 3 patients no discernible pathology was seen. By IF, all 5 patients had light chain restriction (κ x3, λ x2) in proximal tubular epithelium corresponding to crystals or lysosomes. By EM, rhomboid crystals with lattice-like structure were seen in 2 biopsies, while in 3 remaining biopsies only non-specific lysosomal bodies were present in the proximal tubular epithelium. However, by immuno EM, the lysosomal content showed LC restriction (in 2/3 cases studied). On follow-up, 1 of 2 patients with crystals was diagnosed with multiple myeloma, and underwent bone marrow transplant and chemotherapy. Post-transplant, he was found to have isotypic (κ LC) AL amyloid and expired 6 months after the diagnosis of crystalline LCPT. The 2nd patient had a history of analgesic abuse and renal failure for which she underwent renal transplantation. Recurrence of crystalline LCPT was seen in the allograft biopsy several years post transplant. Her bone marrow biopsies continue to be within normal limits, but serum immune electrophoresis shows a discernible κ LC band, 10 years after the initial biopsy. Among the 3 remaining patients, 2 had multiple myeloma (1 had a prior diagnosis, the other was diagnosed within 2 months post kidney biopsy). The third patient has a small isotypic band of monoclonal LC on high-resolution SPEP 2 months post biopsy.

Conclusions: It should be recognized that crystals are not always present, and the presence of lysosomes with LC restriction may be the only clue to the correct diagnosis.

In LCPT, the prognosis is variable and both κ or λ LC may be involved. Our study further expands the spectrum of LCPT.

1220 Lysosomes Are Required by Mesangial Cells To Participate in Renal AL-Amyloidogenesis

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Background: Glomerular amyloidogenesis generally begins in the mesangium and mesangial cells play a fundamental role in this process, presumably engaging in fibrillogenesis occurring in the mature lysosomal compartment. The amyloid fibrils are then extruded to the extracellular matrix where they activate metalloproteinases and eventually replacing the normal mesangial matrix. The present study was conducted to further investigate the role of lysosomes in mesangial amyloidogenesis.

Design: Human mesangial cells (HMCs) were cultured on glass slides and petri dishes, then incubated with light chains (LCs) purified from the urine of patients with AL-amyloidosis (AL-Am) in culture medium for up to 96 hours. HMCs were also incubated with LCs purified from the urine of patients with light chain deposition disease (LCDD) and untreated HMCs used as controls. Lysosomal fractions were prepared from HMCs using buoyant density in a self-generated iodixanol gradient. Immunofluorescence staining was used to analyze the expression of early endosomal antigen (EEA), lysosomal-associated membrane protein (LAMP), and cathepsin-D (the last two present in mature lysosomes) in the HMCs and fractions. Electron microscopic (EM) examination and ultrastructural immunogold labeling were also performed on HMCs and lysosomal fractions to identify amyloid fibrils and precursor protein (LCs).

Results: HMCs incubated with AL-Am-LCs showed abundant expression of LAMP and cathepsin-D and lower expression of EEA. The opposite was noted in HMCs treated with LCDD-LCs and in control HMCs. Significantly higher numbers of lysosomes were also noted in intact HMCs and lysosomal fractions from specimens incubated with AL-Am-LCs as confirmed by EM. Amyloid fibrils were identified associated with and in areas adjacent to lysosomes. Immunogold labeling for light chains detected the precursor protein in association with lysosomes and fibrils in AL-Am-LC treated HMCs. No amyloid fibrils were noted in HMCs incubated with LCDD-LCs and in those cultured without LCs.

Conclusions: This study provides confirmation for the crucial role that lysosomes play in renal AL-amyloidogenesis. Renal amyloidogenesis requires an active participation of mesangial cells, which initially transform from a smooth muscle phenotype to a macrophage phenotype to engage in endocytosis and processing of amyloidogenic LCs.

1221 Glomerular CD99 Staining Is Altered in Acute, Sub-Acute, and Chronic Thrombotic Microangiopathy

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Background: CD99, a 32-kD cell surface molecule, is present on monocytes, most leukocytes, and some tumors. CD 99 has been identified on endothelial cells with highest concentrations at cell-cell borders, playing a critical role in adhesion and the diapedesis of monocytes and neutrophils. Little is known of CD99's distribution or role in the kidney. Thrombotic microangiopathy (TMA) results from endothelial damage from a variety of causes. Characteristic glomerular changes of acute (aTMA), sub-acute (saTMA), and chronic (cTMA) are recognized. We describe staining patterns of CD99 in TMA.

Design: Immunohistochemical (IHC) staining of paraffin sections with a monoclonal antibody to CD99 (Hec-2) was performed on normal kidney tissue from 5 tumor nephrectomies (TNeph), 2 biopsies with thin glomerular basement membranes (TBM), and 3 with minimal change disease (MCD) as controls – normal or diseases without expected glomerular capillary disease – and 18 renal biopsies with TMA: 5 aTMA, 6 saTMA, and 7 cTMA, all previously diagnosed by light, immunofluorescence, and electron microscopy. Each biopsy contained 3 to 22 glomeruli (mean 8.5) for examination and was assigned a pattern of staining: (A) moderate perinuclear/cytoplasmic endothelial/faint luminal staining (B) moderate to strong cytoplasmic endothelial/strong luminal staining (C) Diffuse moderate/finely granular endothelial staining (D) Diffuse strong/coarsely granular endothelial/mesangial staining.

Results: 79% of TNeph glomeruli were pattern (A). 60% of all MCD glomeruli were pattern (A) with 34% pattern(B). 56% of all TBM glomeruli were pattern (A) with 44% pattern(B). 84% of aTMA glomeruli were pattern (C). 68% of saTMA glomeruli were pattern (C) with 32%(B) 9%(D). 90% of cTMA glomeruli were pattern (D).

Conclusions: IHC staining for CD99 produces different patterns in TMA compared to controls. Among TMA biopsies, patterns and staining intensity vary predictably with acute, sub-acute, and chronic changes. CD99 staining in acute endothelial injury (aTMA) has predominantly low-moderate intensity with fine granularity (pattern C). cTMA shows diffuse, high intensity, coarsely granular staining (pattern D). Sub-acute injury (saTMA) shows a mix of patterns (C) and (D). The findings may represent redistribution and/or up regulation of CD99 in response to endothelial injury. CD99 staining is altered/increased with endothelial injury and subsequent healing but studies are needed to fully elucidate the role of CD99 in disease progression.

1222 Chronic Interstitial Inflammation in HIV Patients Has Higher Lymphatic Vessel Density Than Acute Interstitial Inflammation of Allergic Type

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Background: Interstitial inflammation (IN) commonly causes renal dysfunction in many conditions including transplant rejection (TREJ), allergic-type acute interstitial nephritis (AIN), and HIV associated nephropathy (HIVAN). The physiologic and molecular mechanisms that govern the influx and egress of these inflammatory infiltrates

are incompletely understood. TREJ studies have linked nodular infiltrates to lymphangiogenesis (LAG), with increased lymphatic vessel density; they also suggest a special role for both macrophages and podoplanin (PODO), a specific lymphatic endothelial marker, in the evolution of IN. We examined AIN and HIVAN with interstitial infiltrates for similar LAG.

Design: Kidney biopsies from 43 HIV positive and 10 AIN patients were scored for degree of IN (1=minimal, 4=diffuse) Immunohistochemical staining of paraffin embedded sections with monoclonal antibodies to PODO, D2-40 (lymphatic endothelial markers), and KP-1 (macrophage marker) was performed on the 14 HIVAN and 9 AIN biopsies that scored 4 for IN and on normal kidney (control) from 3 tumor nephrectomies. A mean lymphatic vascular density (MLVD), expressed as the number of PODO or D2-40 positive vascular profiles/hpf (40x), was calculated on 5 to 10 consecutive cortical fields per case. The percentage of inflammatory cells positive for KP-1 was scored as 1+(<5%), 2+(5-20%), 3+(20-30%), 4+(>30%).

Results: The MLVD of PODO and D2-40 on all controls was 0.4 (range 0.1 to 0.7) and 0.9 (range 0.8 to 1.0) respectively. The MLVD of PODO and D2-40 for all AIN cases was 1.4 (range 0.3 to 2.6) and 1.5 (range 0.4 to 3.2) respectively. The MLVD for PODO and D2-40 for all HIV cases was 4.3 (range 2.0 to 7.2) and 4.5 (range 2.7 to 7.6) respectively. KP-1 scores for the AIN cases were as follows: four 2+, and five 4+. KP-1 scores for the HIV cases were as follows: two 1+, four 2+, four 3+, and four 4+.

Conclusions: These results indicate that all HIVAN biopsies with diffuse (4+) IN examined had a higher MLVD by both PODO and D2-40 than controls and had higher average MLVD than cases of AIN. AIN biopsies had a slightly higher average MLVD by both PODO and D2-40 than controls but the ranges overlapped considerably. The samples of HIVAN and AIN have similar KP-1 scores suggesting factors other than macrophage density affect MLVD. The chronic nature of the HIVAN infiltrates compared to those in AIN suggests time could promote prolonged lymphangiogenesis possibly leading to subsequent irreversibility of interstitial disease.

1223 Nephron Segment Localization of Polyoma Virus Replicative Activity in Renal Allografts: Quantitative Studies

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Background: Polyoma virus (PV) is a pathogen of the renal allograft associated with significant graft loss. Immunohistochemical colocalization of CD10 in proximal or epithelial membrane antigen (EMA) in distal tubules, with PV large T antigen (TAg), was employed to determine the extent of viral replicative activity in cortical tubular segments.

Design: Serial 2-micron paraffin sections were stained using monoclonal antibodies to PV TAg by standard immunoperoxidase with diaminobenzidine. This was followed by a second immunolabeling step using either CD10 as a proximal nephron marker or epithelial membrane antigen (EMA) as a distal tubular marker, and alkaline phosphatase with fast red. A method of sampling of cortical tubular profiles, at 400x employing an ocular grid, was applied to double-labeled sections of cortex in 6 biopsies with polyoma virus nephropathy (PVN) and 3 allograft controls with histologically normal cortex.

Results: A sample of 2449 and 2560 cortical tubular profiles from biopsies with PVN and 952 and 1179 tubular profiles from controls were sampled on respective CD10TAg and EMATAg double-labeled sections. Of the entire population of TAg+ tubules sampled 16% had CD10 expression and 76.3% had EMA expression. Approximately 7.7% of TAg+ tubular profiles were unidentifiable. The estimated extent of proximal tubular TAg expression (CD10+TAg+/total CD10+) was 5.1%. The estimated extent of distal tubular/collecting ductal TAg expression (EMA+TAg+/total EMA+) was 21%.

Conclusions: These observations demonstrate predominance of distal tubular segment involvement in cortical PV infection and suggest that distal tubular infection precedes infection of proximal tubules. An ascending route of infection of the renal allograft cortex is suggested by these observations.

1224 Glomerular Pathology in Renal Allografts for End-Stage Lupus Nephritis

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Background: The spectrum of glomerular pathologic lesions observed in recurrent lupus nephritis may reflect the spectrum observed in native lupus nephritis.

Design: One hundred-fifty nine biopsy samples from 49 allografts were obtained for dysfunction in recipients with end stage lupus nephritis over a 12 year period. Controls biopsies (n=120), were from patients with grafts for diseases other than lupus (n=47), matched for recipient age, and obtained for graft dysfunction in the same time period after transplantation. Biopsy specimens were examined by standard light, immunofluorescence and electron microscopy.

Results: Glomerular pathology was observed in 16 allograft biopsies 1.2 to 83 months (mo) (median 17.5 mo) after transplantation for end stage lupus nephritis in 10 patients. Focal necrotizing glomerulonephritis was observed in 5 of 16 at 5-27 mo and 2 had mixed necrotizing and sclerosing lesions at 7 and 23 mo. Five of 7 had prominent IgM and fibrin with little detectable IgG and ultrastructural electron dense deposits were sparse. Four had de novo collapsing glomerulopathy with podocytopeny identified at 1-36 mo. Atypical membranous lesions were observed at 60 and 83 mo and mesangial-limited immune deposits were observed in 3 biopsies obtained more than 36 mo after transplantation. These lesions were absent from the controls.

Conclusions: Focal IgM-associated necrotizing and sclerosing lesions and podocytopeny were the most frequently observed glomerulopathies in lupus recipients. Glomerular pathology unique to allograft recipients with lupus presents a spectrum that does not readily fit current classifications of lupus glomerulonephritis in the native kidney.

1225 A Murine Model of Acute Humoral Rejection in Renal Allografts

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Background: Acute humoral renal allograft rejection (AHR) is gaining increasing attention because of its frequently poor outcome and because of the introduction of C4d staining of renal allograft biopsies, which greatly facilitates the diagnosis of AHR. In AHR, the peritubular capillaries (PTC) show diffuse staining for C4d and frequently also for C3d. By light microscopy, the PTC and the glomerular capillaries contain many inflammatory cells, there may be interstitial hemorrhage and, in the more severe cases, glomerular or arteriolar fibrin thrombi may develop. It is common to see some degree of cellular (interstitial) rejection combined with AHR. AHR is particularly common in recipients who are presensitized to donor antigens. The purpose of our project was to develop an easily reproducible animal model for AHR.

Design: We have previously shown spontaneous mouse renal allograft acceptance in the DBA/2 to C57BL/6 strain combination. In the current experiments we placed DBA/2 skin allografts on C57BL/6 mice and allowed rejection within 12 days. Following skin rejections, five C57BL/6 mice received DBA/2 renal allografts. The native kidneys of the recipient mice were removed. Between 5 to 19 days after renal transplantation, the recipient mice started to display renal dysfunction and were sacrificed.

Results: Histology of the renal allografts revealed prominent margination of inflammatory cells in the PTC, mild to moderate diffuse interstitial mononuclear cell infiltrate with mild and rare tubulitis, mild to moderate interstitial edema with mild patchy interstitial hemorrhage and deposition of amorphous material (probably fibrin) in many glomerular capillaries. Indirect immunofluorescence for C3d revealed diffuse PTC fluorescence in all renal allografts. All recipient animals had high levels of anti-donor antibodies. The anti-donor antibody levels that developed following the skin graft rejection further increased after the kidneys were transplanted.

Conclusions: The clinical setting and histologic findings in our model strongly resemble AHR in human renal allograft recipients with preexisting donor specific antibodies. This animal model enables us to study the pathogenesis of AHR and the effects of potential therapeutic interventions.

1226 Podocyte PAI-1 Affects AngiotensinII(AngII)-Induced ECM Accumulation

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Background: AngII induces collagen synthesis, and also upregulates PAI-1 through the angiotensin type1 receptor (AT1R) by non-pressure dependent mechanisms. However, the interactions of AngII and PAI-1 on podocyte pathobiology is not well known. We investigated whether PAI-1 modulates AngII-induced collagen synthesis in podocytes in vitro.

Design: Glomeruli were harvested from WT and PAI-/- C57/bl mice by sieving. Podocytes were isolated and subcultured on collagen type 1 coated plates, and identified as podocytes by positive staining for synaptopodin and WT-1. Podocytes were then left untreated or treated with AngII (10⁻⁷M) for 48hrs in the absence or presence of antagonist for the AT1R (AT1RA, 10⁻³M losartan) or AT2R (AT2RA, 10⁻⁵M PD123319) or combination(losartan+PD123319). We evaluated expression of collagen α 1(IV) by immunofluorescent staining, analyzed by Scion Image software, expressed as % field occupied by staining and normalized to control.

Results: In WT podocytes, AngII significantly increased the level of collagen α 1/2(IV) compared to control (158.0 \pm 1.9% of control, p<0.05). AT1RA markedly decreased AngII-induced collagen with lesser but still significant decrease with AT2RA or combination (AngII+AT1RA 65.5 \pm 11.3, AngII+AT2RA 97.9 \pm 19.7, AngII+combination 81.2 \pm 20.5% all vs control, AngII vs AT1RA or combination p<0.01, AngII vs AT2RA p<0.05). In contrast, AngII did not induce collagen in PAI-/- podocytes, and neither AT1RA nor AT2RA had significant effects.

Conclusions: In conclusion, podocyte fibrotic response to AngII is largely AT1-mediated, and is dependent on the presence of PAI-1.

1227 Thrombomodulin but Not Endothelial Cell Protein C Receptor Expression Is Reduced in the Tubulointerstitial Capillaries of Patients with Proliferative and Membranous Lupus Nephritis

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Background: Thrombomodulin (TM) and the endothelial cell protein C receptor (EPCR) are the key endothelial cell receptors of the protein C (PC) anti-inflammatory and anticoagulant pathway. Down-regulation of TM and/or EPCR may suppress the anti-inflammatory, antiapoptotic, and anticoagulant signaling and may augment the inflammatory response. To test the hypothesis that TM and/or EPCR expression might be reduced in the tubulointerstitial (TI) capillaries of patients with lupus nephritis (LN) we have quantitatively analyzed endothelial TM and EPCR expression in the TI capillaries as visualized by immunohistochemistry in renal biopsies with proliferative or membranous LN.

Design: Formalin-fixed paraffin-embedded sections of 19 renal biopsies from patients with systemic lupus erythematosus (SLE) (mean age 31.5 years; range 12-62 years) showing proliferative [WHO class III or IV (n=10)] or membranous [class V (n=9)] LN were stained immunohistochemically for CD34, TM and EPCR. Controls included 7 patients (mean age 21.8 years; range 10-46 years) with minimal change nephrotic syndrome without clinical history of SLE. The microvessel density (MVD) of the CD34-, TM-, and EPCR-positive TI capillaries was determined in each biopsy. Microvessel counts for all three stains were performed manually at 200x magnification on five digital images of each biopsy using a 600-point grid mounted digitally over the photographs. Student t test was used to assess significant differences of MVDs for each marker between various groups.

Results: CD34-stained slides showed the highest MVD in controls (0.0710±0.04) followed by TM (0.0257±0.013) and EPCR (0.0106±0.014). In proliferative and membranous LN cases the MVD was not significantly different for CD34 and EPCR from the control groups. However, TM showed significantly lower MVD in both the proliferative (0.0107±0.009; $P=$.020) and membranous (0.0059±0.004 $P=$.005) LN cases as compared with controls.

Conclusions: Selective down-regulation of TM expression in the renal TI capillaries of patients with proliferative and membranous LN raises the possibility of differential *in vivo* regulation of the TM and EPCR genes. Down-regulation of TM expression points to endothelial dysfunction that may augment the TI inflammatory response and ensuing tissue injury.

1228 Detection of T Regulatory Cells Expressing Foxp3 in Renal Allografts: Potential Pathogenetic and Diagnostic Implications

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Background: Foxp3 is a transcription factor inducible by TGF β and expressed specifically by T regulatory cells (Treg), which downregulate the immune response. We have examined renal allografts for the expression of Foxp3 to determine whether these cells may have pathogenetic or diagnostic significance.

Design: Renal allograft biopsies with a diagnosis of acute rejection or calcineurin inhibitor toxicity (CIT) and biopsies of living donor kidneys at transplant were studied. Sections from paraffin blocks were coded and stained with a polyclonal rabbit anti-Foxp3 peptide (Abcam) or anti-CD3 (DAKO) with immunoperoxidase techniques. Positive cells in the cortex were counted for the entire section and the results expressed as +cells/mm² cortex and as the ratio of Foxp3+/CD3+ cells.

Results: Foxp3 was detected in nuclei of infiltrating mononuclear cells and rarely in cells in capillaries. Foxp3+ cells were most prominent in the interstitial infiltrate of grafts with acute rejection, particularly among aggregates of T cells, averaging 27% of the CD3+ T cells. Foxp3 cells were also present occasionally in the tubules (tubulitis) in ACR. Foxp3 cells were much less common in CIT and normal kidneys (Table). There was no difference in C4d+ vs C4d- biopsies with acute rejection. However, biopsies with acute rejection that had a poorer outcome after the biopsy ($Cr \geq 2.0$ at 12 mo) had fewer Foxp3 cells per CD3 cell than those with a better outcome ($Cr < 2.0$ at 12 mo).

Conclusions: These results indicate that Treg cells, as defined by expression of Foxp3, are a substantial fraction of the T cells in acute rejection. Treg may be induced locally by TGF β , known to be produced by tubular and other cells. The presence of Treg cells argues that the infiltrate termed "rejection" may be generating immune inhibitory processes in addition to graft injury. Paradoxically, Treg "tubulitis" may serve to downregulate the T cell response.

Diagnostic Group	N	Table		
		Foxp3/mm ²	CD3/mm ²	Foxp3/CD3 (%)
Acute Rejection	21	8.2±6.6*	73.7±80.7	27.2±33.8*
C4d-	12	8.9±6.2	92.4±89.1	26.6±34.0
C4d+	7	6.7±7.5	36.2±45.3	28.4±36.1
12 mo Cr<2.0	12	8.8±6.2	78.6±100	39.1±39.6**
12 mo Cr≥2.0	7	7.4±7.3	67.0±48.9	11.4±14.5
CIT	7	1.9±3.0	44.4±30.6	4.8±6.1
Donor Kidneys	4	0.7±1.0		

* $p < 0.04$ vs CIT or donor biopsy; ** $p < 0.05$ vs $Cr \geq 2.0$; Student's test.

1229 Peritubular Capillary C4d Deposition in Chronic Renal Allograft Rejection – How Relevant Is It?

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Background: Detection of peritubular capillary (PTC) C4d deposition in tissue sections of renal allograft biopsies has become an important aid in the diagnosis of humoral rejection. Evidence shows that diffuse C4d deposition along PTC endothelium, as detected by immunostaining is an adverse prognostic factor for graft outcome in early acute rejection. However the clinical significance of C4d deposition in late acute and chronic rejection is still confusing and largely unknown. We examined graft outcomes in subsets of C4d positive and C4d negative late acute/chronic rejection cases and cases with chronic allograft nephropathy with and without C4d, excluding cases with early acute rejection.

Design: We retrieved renal allograft biopsies taken more than 12 months post transplant from our pathology archives (2003 to 2005, after the introduction of routine C4d staining). The patients were classified into 4 groups: 1. C4d+ late acute interstitial rejection (LAR) (n=33), 2. C4d+ chronic allograft nephropathy (CAN) without LAR (n=14), 3. C4d- LAR (n=23), and 4. C4d- CAN without LAR (n=13). Groups were matched by average CADI scores, and number of months from transplant to end of follow-up (F-U) period. Histologic features, percent graft loss, serum creatinine levels at last followup, were studied and compared between the 4 groups.

Results: Rate of renal allograft loss was 39% in the group with C4d+ LAR versus 52% in C4d- LAR. Among the groups with CAN and no evidence of LAR, the C4d+ and C4d- groups had graft loss rates of 42% and 46% respectively. There were no significant differences in the average last F-U serum creatinine levels between any combination of the 4 groups (ANOVA, $p=0.373$, 0.514, 0.416, and 0.48 respectively). Average F-U period after biopsy was 11.7 months. Among the histologic features, chronic transplant glomerulopathy was present in 36% of cases with C4d+ LAR versus 8% of the cases with C4d- LAR (Fisher Exact test, $p < 0.05$); and 64% of C4d+ CAN cases versus only 15% of the C4d- CAN cases ($p < 0.05$).

Conclusions: Although the follow-up period is short, our study does not support a deleterious effect of PTC C4d deposition in late acute and chronic humoral rejection which is in sharp contrast to published results in early acute rejection of renal allografts. However our study does confirm that transplant glomerulopathy is significantly associated with PTC C4d deposition in late acute and chronic humoral rejection.

1230 Pathogenesis of Extra Efferent Vessel (EEV) Development in Diabetic Glomeruli

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Background: Extension of our study (Lab Invest 80:178A, 2000) combined with data from the only other large study known to us (Virch Arch A Path Anat 423:201, 1993) confirm that extra efferent vessels (EEV) develop in diabetic glomeruli. In our material up to 18 EEV were found in diabetic glomeruli, and the number of EEV was associated with the severity of diffuse and nodular glomerular mesangial expansion. In our opinion the development of EEV represents a remarkable remodelling of the glomerulus that should be added to the list of changes that occur in diabetic glomerulopathy. This report presents a proposed pathogenesis for the development of EEV.

Design: PAS stained step serial and serial sections, plastic embedded 1 micron serial sections with complete glomerular reconstructions of 1 glomerulus from each of 6 cases, and 7 step serial section electron microscopic whole glomerular montages of 1 glomerulus were studied in 18 mostly Type II diabetic cases with mild to severe (but not end stage) diffuse and nodular mesangial expansions, 8 matched controls, and 2 normal traumatic nephrectomy specimens from an 18 and a 39 year old male, respectively.

Results: EEV exited the glomerulus adjacent to vascular pole structures through small gaps in the glomerular capillary and/or Bowman's capsule basement membrane (BM). These little known BM gaps, easily seen only in plastic embedded 1 micron sections, were well defined at the edges of the vascular pole (VP), but were also present centrally. The gaps were thought to be preexisting because similar gaps, without EEV, were found in all cases. Mean counts of gaps at the edge of the VP (with and without EEV) in 5 glomeruli from each of 6 cases (2 traumatic nephrectomy controls-age 18 and 39, 2 older controls-age 60 and 60, and 2 diabetics-age 29 and 75) gave the following results, respectively: 1.4 (0-3), 0.8 (0-3), 4.6 (2-8), 6.8 (4-13), 4.4 (3-6) and 11.2 (4-18). In many gaps without EEV only a thin layer of endothelium and mesangium separated the capillary lumen from the renal interstitium or the VP structures.

Conclusions: The very development of EEV suggests that increased glomerular outflow is needed and thus implies the presence of chronically increased glomerular flow and/or pressure. The walls of EEV consist of endothelium and mesangium without a glomerular capillary BM. These factors led to the hypothesis that increased glomerular flow and/or pressure pushing against the thin layers of endothelium and mesangium covering the gaps could initiate EEV formation.

1231 Collapsing Glomerulopathy: Structural Progression as Delineated by Repeat Renal Biopsies

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Background: Collapsing glomerulopathy, a pan-nephropathy with primary epithelial cell damage, is considered a form of focal and segmental glomerulosclerosis. Clinically it presents with heavy proteinuria and acute/progressive renal failure culminating in end stage renal disease in up to 75% if untreated. Consequently, repeat renal biopsies are very infrequently performed and morphologic evolution has not been studied in detail.

Design: Renal biopsies of 3 patients (2 males, 1 female) aged 2 years 5 months, 16 years and 40 years respectively presenting with heavy proteinuria, with 1 also manifesting renal insufficiency (serum creatinine 1.7) were evaluated by LM, IF, EM, and IP for parvovirus B19. After 8, 41 and 56 months, 2 had second biopsies because of persistent proteinuria and renal insufficiency despite initial improvement in one patient; while the 3rd with steroid dependent nephrotic syndrome underwent a repeat biopsy for evaluation of possible cyclosporine nephrotoxicity. All were HIV negative and were not receiving bisphosphonate therapy. One patient had end stage lupus nephritis and developed collapsing glomerulopathy in the transplanted kidney.

Results: Initial biopsies disclosed hypertrophy, hyperplasia and coarse vacuolization of visceral epithelial cells in 12% to 25% of glomeruli. Diminished brush border staining, irregular flattening and mitotic figures in proximal tubular cells were observed in 2 biopsies. In the repeat biopsy in the transplant patient 8 months later, the glomerular changes were identical, involving 50% of glomeruli, as were tubular alterations. In the other patients, glomerular abnormalities progressed to typical well formed segments of sclerosis and affected 10% in one and 6% in the other. No lesions of acute tubular necrosis were evident; instead, there was focal tubular atrophy with interstitial fibrosis. Parvovirus B19 antigen was not demonstrated.

Conclusions: Collapsing glomerulopathy may remain in a morphologically active state for many months or may progress to typical focal and segmental glomerulosclerosis in patients who have persistent proteinuria and renal functional impairment.

1232 Effect of Dietary Phosphate and Dehydration on Renal Function in ACE-Inhibitor Treated Diabetic Rats

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Background: Decline in kidney function is prevalent in patients with impaired renal blood flow, such as in diabetic and hypertensive patients. The mechanisms leading to decline in renal function in diabetic patients on ACE-inhibitors after administration of oral phosphate is poorly understood. We investigated whether dehydration impairs renal function in diabetic animals treated with an ACE-inhibitor and fed on high phosphate diet.

Design: Male Sprague Dawley rats were divided in eight groups (A-H) with 6 animals per group. Each animal in A-D was injected with a single dose of 60 mg streptozotocin. Groups B and C received enalapril (100 mg/L) in drinking water. Nondiabetic control groups F and G also received enalapril. On day 8 of experiment each of the eight groups were further divided into two subgroups (high and low phosphate diet). On day 25 the experiment groups C, D, G and H were dehydrated for 72 hours. On day 28 all animals were sacrificed. Blood, urine and kidney tissue samples were collected.

Results: Streptozotocin treated animals showed doubling of serum glucose levels by day 14, which remained elevated until day 28. Diabetic animals, treated with ACE-

inhibitor and fed with high phosphate diets showed marked increases in BUN levels, which was further augmented following dehydration. Creatinine levels also dramatically increased in ACE-inhibitor treated diabetic animals following dehydration. No significant changes in urine or blood pH values were seen in diabetic or non-diabetic animals with or without ACE-inhibitor or high phosphate diet. No calcium phosphate crystals were seen either on H & E or von Kossa stained kidney tissue sections. However, tissue sections of dehydrated diabetic animals on high phosphate diet treated with ACE-inhibitor showed acute tubular necrosis.

Conclusions: In summary our study shows that in diabetic rats the combination of ACE-inhibitor therapy, dehydration and high phosphate diet may predispose to acute tubular necrosis. This observation may have significant public health implications since the number of diabetic patients is increasing world wide and ACE-inhibitors are commonly used in these patients to treat high blood pressure. Furthermore, a large percentage of these patients might consist of elderly diabetics who are known to be prone to dehydration for various reasons. Further studies are indicated to investigate on a cellular level the underlying mechanisms that lead to tubular epithelial cell necrosis in these patients.

1233 Kidney Injury Molecule-1 (KIM-1) Is a Specific and Stable Target for Identifying Proximal Tubular Injury in Humans

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Background: We have reported that KIM-1 is a specific injury biomarker for proximal tubules in several animal models and a limited number of human kidneys with tubular injury. We have found that the *Kim-1* gene is markedly upregulated together with a group of stress proteins (among 31,100 genes) at an early phase of renal ischemia-reperfusion injury in rats. This study was performed to further evaluate the expression of KIM-1 in several groups of human renal diseases.

Design: Groups consisted of normal kidneys examined away from renal tumors in nephrectomy specimens, protocol renal transplant biopsies, renal transplant biopsies with mild or moderate acute tubular necrosis (ATN), acute cellular rejection (ACR), and varying types of glomerulonephritis (GN) (total n = 93). In addition kidney sections from 19 autopsy cases were randomly identified. Paraffin embedded tissue sections were stained with monoclonal antibody against human KIM-1 using a DAKO Autostainer. The intensity score of KIM-1 and MIB-1 positive staining (along the luminal surface of proximal tubules and in nuclei, respectively) was graded from 0 to 3 (0, no staining; +/-, focal granular staining; 1+, weak granular staining; 2+, moderate granular staining and 3+, strong granular staining). Focal staining (+/- category) was scored as a positive case.

Results: In protocol biopsies of transplanted patients there was a low rate of positive staining for KIM-1 (see table). Kidneys from patients with a clinical diagnosis of mild ATN were positive 46% of the time, but moderate ATN, confirmed by pathology, was associated with 100% positivity. ACR and GN cases also had a high rate of positive KIM-1 staining. In contrast, these groups all had low positive staining rate of MIB-1 (Ki-67). In addition, cadaver kidneys with ATN showed positive KIM-1 staining in proximal tubules, despite prominent autolysis.

Conclusions: Our data indicate that KIM-1 is specific marker which can be used to indicate proximal tubular injury with varying types of renal insults and appears more sensitive than MIB-1 to highlight the injury. Furthermore, KIM-1 is a stable target that is identified even in injured kidneys with prominent autolysis.

	KIM-1 and MIB-1 Expression in Human Renal Tissue.					
	Normal Kidneys	Protocol biopsies	Mild ATN	Moderate ATN	ACR	GN
KIM-1 positive	1/13	5/24	6/13	8/8	11/12	18/23
MIB-1 positive	0/13	2/24	0/13	1/8	1/12	5/23

Liver & Pancreas

1234 Von-Meyenburg Complexes Increase with Age and Are Specifically Associated with Alcoholic Cirrhosis and End-Stage Hepatitis B Infection

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Background: Biliary hamartomas or Von-Meyenburg complexes (VMCs) form part of a spectrum of ductal plate abnormalities that includes polycystic liver disease, congenital hepatic fibrosis, and Caroli's disease. VMCs are usually regarded as common and innocuous lesions, but cholangiocarcinoma (CCA) is known to be increased in patients with ductal plate abnormalities. Additionally, there are now a small number of case reports and series that link CCA to a background of multiple VMCs. Because cirrhosis, alcohol, and viral hepatitis have been epidemiologically linked to intrahepatic CCA, we evaluated the prevalence and associations of VMCs in a large series of liver explants.

Design: We studied 567 liver explants performed for cirrhosis due to Hepatitis C (HCV) (n=154), alcohol (n=112), HCV/alcohol (n=85), Hepatitis B (HBV) (n=67), and other (excluding chronic biliary tract disease) (n=149). Controls included 134 explants in non-cirrhotic conditions (e.g., metabolic abnormalities, acute liver failure). None had polycystic liver/kidney disease or congenital hepatic fibrosis. For each case, we recorded age, gender, number of VMCs, and number of histologic sections available. Statistical analysis included chi-square test for categorical data, t-test for non-categorical data, and logistic regression for multivariate analysis.

Results: VMCs were identified in 126 of 701 (18%) livers. Number of slides reviewed did not differ significantly between cases with and without VMCs (5.5 vs 5.0, p=0.07). In univariate analysis, older age (p<0.0001), male gender (p<0.0001), and cirrhosis of any type (p=0.012 after correcting for age and gender) were all correlated with prevalence of VMCs. In multivariate analysis, only age (p<0.0001) and cirrhosis from alcohol

(p=0.001), HBV (p=0.001), and HCV/alcohol (p=0.013) were significant. VMCs were seen in only 1 of 171 (0.6%) patients \leq 35 years of age. Among patients with alcoholic cirrhosis, VMCs were present in 15% overall and 5 (4.5%) had numerous (>10) lesions (range 11-98). One of these 5 also had multifocal intrahepatic papillary bile duct dysplasia.

Conclusions: Despite their apparent hamartomatous appearance, VMCs outside the setting of congenital hepatic fibrosis or polycystic liver disease are acquired lesions that increase with age and are associated specifically with cirrhotic-stage liver disease due to alcohol, HBV, and HCV/alcohol. A minority of patients with alcoholic cirrhosis have numerous VMCs resembling a "forme fruste" of congenital ductal plate abnormality.

1235 Mucinous Nonneoplastic Cysts of the Pancreas: Clinical and Immunophenotypic Analysis

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Background: Mucinous nonneoplastic cyst (MNC) is a recently described cystic lesion of the pancreas characterized by mucinous differentiation of the lining epithelium, a thin rim of almost acellular supporting stroma, and the absence of communication with the pancreatic ducts. MNC is difficult to distinguish from mucinous cystic neoplasms on clinical and radiographic grounds and as a result is often surgically resected. In addition, it is unclear whether MNC represents a precursor lesion to pancreatic carcinoma or truly a nonneoplastic cystic change. In an attempt to better understand the nature of MNC, we examined clinical and morphologic data and MUC1, MUC5AC, p16 and DPC4 expression in 12 patients with MNC.

Design: Review of archival material from the surgical pathology files revealed 12 cases of MNC that met previously established histologic criteria. All available clinical data was reviewed including aspirated fluid CEA level if available. Immunohistochemistry for MUC1, MUC5AC, p16, and DPC4 was performed on paraffin-embedded, formalin-fixed tissue using monoclonal antibodies and standard avidin-biotin technique. The percentage of positive cells was estimated for all stains.

Results: The patients included 8 females and 4 males ranging from 20 to 80 years of age. Six lesions were grossly unilocular, one was bilocular, and 5 were multilocular. Size ranged from 0.6 cm to 9.0 cm. Three cysts were localized to the head, two to the body, and seven to the pancreatic tail. In all ten patients where cystic fluid was analyzed for CEA, the levels of this marker were high and ranged from 160 to 11321 ng/ml (mean 2731 ng/ml). H&E stained sections showed that the cysts were lined by a single layer of mucinous epithelium without any cytological atypia or mitosis. Nuclear expression of p16 and DPC4 was observed in all the cysts. MUC5AC and MUC1 expression was observed in 10 (83%) and 4 (33%) of cysts, respectively.

Conclusions: Morphologic and molecular studies support the nonneoplastic nature of these mucinous cystic lesions of the pancreas. Proper classification and identification of these cysts is needed to obviate unnecessary surgery. CEA levels of the cystic fluid are high in MNC and should not be used to differentiate these cysts from other mucinous cystic neoplasms.

1236 Adenocarcinoma of the Minor Duodenal Papilla and Its Precursor Lesions: A Clinical and Pathological Study

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Background: The minor duodenal papilla, which drains the accessory pancreatic duct (of Santorini), lies proximal to the ampulla of Vater. Unlike in the ampulla and elsewhere in the duodenum, adenocarcinoma and its precursor lesions arising in the minor papilla are rare and consequently poorly defined.

Design: Tumors occurring in the duodenum proximal to the major papilla that were treated at our institution and had sufficient material were reviewed. Those fulfilling the following criteria were selected and regarded as tumors of the minor papilla: location at 1.5 cm - 2.5 cm proximal to the major papilla; presence of associated submucosal pancreatic ducts with peri-ductal glands and/or acinar tissue; a predominant submucosal location of the tumor; and lack of adenoma in the adjacent duodenal mucosa. Tumors thus identified were studied morphologically, immunohistochemically and clinically.

Results: Five cases of adenocarcinoma arising in the minor papilla were identified among 17 supra-ampullary carcinomas (29%). The results are summarized in the table.

Case no.	Age/Sex	Size	Precursor (type)	Invasive Carcinoma (type)	Pathologic Stage	Follow up (months)	Survival Status
1	75 / M	3.0 cm	IPMN*	Colloid	pT4N0M0	75	DOD
2	77 / F	1.8 cm	IPMN	Colloid	pT2N0M0	13	NED
3	77 / M	1.2 cm	IPMN	Colloid	pT4N0M1	45	AWD
4	71 / F	4.0 cm	Absent	Intestinal	pT4N0M0	85	DOD
5	50 / F	3.0 cm	Absent	non-mucinous Pancreato-biliary	NA	NA	NA

*intraductal papillary-mucinous neoplasm, in all cases with high grade dysplasia

Immunohistochemically, the intestinal and mucinous type tumors were positive for CK20, CDX2, DPC4, MUC2, B72.3, MLH1 and MSH2 and were negative for CK7, MUC1, p53, DUPAN-1 and CA125. Stains could not be performed for case 5.

Conclusions: Adenocarcinomas of the minor papilla are rare tumors occurring predominantly in the 6-7th decade. They may arise from IPMN precursors in the residual submucosal pancreatic tissue. Morphologically, immunohistochemically, and clinically they are similar to ampullary or IPMN-associated carcinomas and can show either intestinal-type or pancreatobiliary-type features. The proximal location with respect to the major papilla, predominantly submucosal location and presence of underlying pancreatic tissue are clues to the diagnosis of these tumors.