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

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

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

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

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

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

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

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

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

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

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

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

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

Liver & Pancreas

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

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

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

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

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

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

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

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

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

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

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

1358 Peripheral Liver Histology in Autoimmune Pancreatitis with Sclerosing Cholangitis (AIP-SC): Is Percutaneous Liver Biopsy Useful?

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Background: Autoimmune pancreatitis (AIP) is a special type of chronic pancreatitis distinct from primary sclerosing cholangitis (PSC). AIP most commonly involves the head of the pancreas and the distal bile duct. When AIP involves the extrahepatic bile ducts, it is felt to represent a secondary form of sclerosing cholangitis (SC). Unlike PSC, AIP responds to steroid therapy. Few reports describe the peripheral liver parenchyma in AIP-SC, and are based on liver biopsy material subject to sampling effect. We evaluated the liver parenchyma in 2 cases of AIP-SC where liver resection was performed due to radiologic suspicion of malignancy.

Design: The study cases are 2 right hepatic lobe resection specimens. Sections of liver, including peripheral liver, are stained with a standard panel of special stains, and cytokeratin 7 immunoperoxidase stain. The control group consisted of 12 explanted livers with PSC. Histopathologic features of parenchymal inflammatory activity, biliary disease, and the morphological features of AIP in pancreas (periductal infiltration by lymphocytes and plasma cells, granulocytic epithelial lesions with focal destruction of the duct epithelium, venulitis) were recorded.

Results: In AIP-SC, the portal inflammation is mild, lymphoid with few plasma cells; similar in that regard to the PSC cases. The exception is that in the AIP cases, portal eosinophils are relatively more frequent, and easily found in the portal tracts of the AIP-SC patients (0-8 cells/portal area), compared to PSC (0-2 cells/portal area). Features of long-standing cholestasis and advanced fibrosis are present in the PSC controls but not the AIP cases. Bile duct loss, periductal sclerosis, and changes similar to those seen in the pancreatic ducts in AIP are not present.

Conclusions: The peripheral liver portal findings in AIP-PSC are mild and nonspecific. Bilirubinostasis related to duct obstruction is present but features of chronic biliary disease are not seen. The latter may be related to shorter duration of disease compared to PSC. The periductal sclerosis reported by others in AIP-SC is not seen in our 2 cases. In our cases, the location of the liver sections is known. In previous studies, the exact location of the liver biopsies is not given; the biopsies could have originated more centrally. Periductal changes reported in these studies may reflect effects of large duct obstruction rather than features inherent to AIP-SC. Percutaneous liver biopsy does not appear to have a role in the diagnosis of AIP-SC.

1359 Proposal of Progression Model for Intrahepatic Cholangiocarcinoma: Clinicopathologic Differences between Hilar Type and Peripheral Type

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Background: It is important to clarify the histologic progression of intrahepatic cholangiocarcinoma (ICC) in consideration of its origin from the intrahepatic large or small biliary ducts.

Design: On the basis of the gross and histologic assessment, we classified 87 cases of ICC smaller than 5 cm in diameter into hilar type (H-ICC, n=38, Fig 1A,B) or peripheral type (P-ICC, n=49, Fig 1C,D) to compare their clinical and histologic features.

Results: Biliary dysplasia was observed in 65.8% (25/38) of H-ICC cases, whereas hepatitis virus infection and liver cirrhosis were associated with 46.7% (21/45) and 28.6% (14/49) of P-ICC, respectively. The frequency of perineural invasion, lymph node metastasis, and extrahepatic recurrence of H-ICC was significantly higher than that of P-ICC (P<0.0001, 0.0106, and 0.0279, respectively). H-ICC cases showed frequent vascular invasion and intrahepatic metastasis even with small tumor size, compared with P-ICC cases. H-ICC showed large duct involvement within the tumor, and in the cases of large tumor size, intraductal spread was detected in the tumor periphery. P-ICC of small size contained preserved architecture of the portal tracts. The survival of patients with H-ICC was worse than that of patients with P-ICC (P=0.0121). The independent and best prognostic factor by multivariate analysis was intrahepatic metastasis for H-ICC and lymph node metastasis for P-ICC.

Conclusions: Our results suggest that ICCs derived from a different level of biliary ducts were related to different premalignant conditions and different tumor progression. Some ICCs arising from the large biliary duct are likely to exhibit an aggressive course even in cases of small tumor size. The recognition of the above events induces the proper therapy.

Figure 1: Gross and histologic appearance of ICC

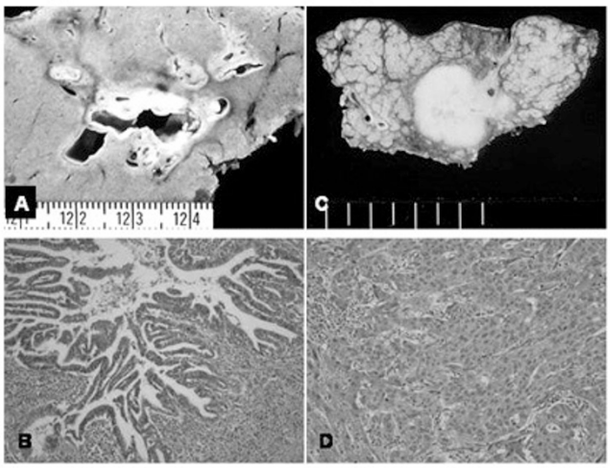
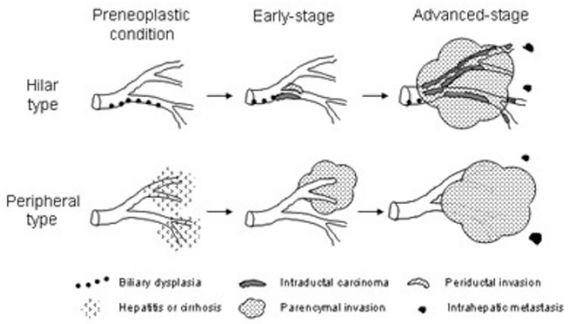


Figure 2: Proposed progression model of ICC



1360 Primary Gallbladder Sarcoma: 18 New Cases

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Background: Primary sarcoma of the gallbladder (PGBS) is rare, with only 38 documented cases, mostly leiomyosarcoma.

Design: Cases recorded as "gallbladder sarcoma" were pulled from our files; the clinicopathologic features were tabulated. Only primary gallbladder wall mesenchymal tumors were included. Epithelial tumors, tumors extending into the gallbladder from the abdomen or sarcoma with other known primaries were excluded.

Results: PGBS occurred in 6 males and 12 females with a median age of 68 (range, 1.5-88) years, including three age 1.5 to 3 with rhabdomyosarcoma. Patients presented with acute or chronic cholecystitis, abdominal pain, weight loss, pruritis, elevated alkaline phosphatase and bilirubin, and leukocytosis. Tumors ranged from 1.5 to 20.0 cm with a median size of 4.0 cm. Most PGBS involved the whole wall and ulcerated the mucosa of the body; three were of fundus/ampulla. PGBS were diagnosed as 9 myxofibrosarcomas (malignant fibrous histiocytoma, MFH, storiform pleomorphic to myxoid, two with an Evanslike and Pleomorphic Hyalinizing Angiectaticlike (PHAT) mixture, 2 leiomyosarcomas (LMS), 1 gastrointestinal stromal tumor (GIST), 3 botryoid embryonal rhabdomyosarcomas (RMS), 1 mesenchymal chondrosarcoma, and 2 angiosarcomas (AS). Diagnosis was based on morphology and immunohistochemistry. LMS required myoid intersecting fascicles and diffuse strong SMA +/- desmin. RMS revealed myxoid grapelike hypocellular tumor with stellate cells, mild atypia, mitoses and desmin and myoregulatory proteins (MyoD1 and myf4) reactivity. GIST was palisaded and myoid-like with CD34/CD117, and AS an extravascular vascular proliferation, atypia, mitotic activity, and CD31 positivity. All patients received cholecystectomy and 6 known adjuvant therapy. Follow-up on 15 revealed that 10 patients died of disease, 3 weeks to 3 years after diagnosis, 3 died of unrelated causes and 2, both adjuvant therapy treated botryoid RMS in young children, were alive without disease 11 and 27 years later.

Conclusions: PGBS are rare. These mainly occur in the gallbladder body in middle aged females with acute cholecystitis, with overall poor prognosis. A variety of sarcoma types are found, yet MFH is the predominant variant, two with a distinctive cross between PHATlike and Evanslike morphology. GIST can exceptionally occur in this location. Adjuvant therapy-treated botryoid embryonal RMS in the gallbladder of young children can have excellent prognosis.

1361 Lipomatous Hypertrophy of the Pancreas: A Clinicopathologically Distinct Entity

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Background: Cases with the clinical diagnosis of pancreatic cancerthat prove to have benign lesions by histologic examination illicit substantial anxiety both for pathologists and the primary physicians. One such condition which is poorly defined and inadequately

documented in the literature, represented only by individual case reports is referred as lipomatous pseudohypertrophy of the pancreas.
Design: In the authors' files, 4 cases of pancreatic tumor composed of mature adipose tissue were identified and their clinicopathologic features analyzed.
Results: The patients were 2 male and 2 female, with ages of 47, 59, 67 and 67. One patient presented with history of chronic pancreatitis and biliary strictures. In the remaining 3, the tumors were detected incidentially during work-up for other conditions. Three patients were operated with the clinical diagnosis of a solid pancreatic tumor, with the main differential diagnosis of ductal adenocarcinoma. In 1 case, however, an expert radiologist correctly interpreted the process to be adipose tissue by MRI, but the patient underwent resection because of the symptoms. 2 tumors were located in the head, 1 in the tail and in 1 case, there was more diffuse involvement of the pancreas. One patient had a separate microcystic adenoma. All patients underwent resection. One underwent a second look operation because the primary physician was convinced that there was a real tumor. The tumors were 24, 7, 13 and 2.5cm in largest dimension. The tumors were composed of mature adipose tissue replacing the pancreatic parenchyma, leaving only scattered clusters of pancreatic elements, (without showing any stigmata of chronic pancreatitis). Pancreatic elements were identified even in the remote peripheral edges of the lesion, and in many areas the tumor was sharply demarcated from the surrounding tissue. No lipoblasts were identified, lipocytes were entirely normal appearing. There was no significant inflammation.
Conclusions: Lipomatous hypertrophy of the pancreas is a distinct entity that is characterized by replacement of pancreatic parenchyma with mature adipose tissue. It forms a pseudotumor that may be difficult to distinguish clinically from pancreatic adenocarcinoma and the patients undergo pancreatic resection. It is possible that these lesions are benign adipocytic neoplasms, i.e intrapancreatic lipomas.

1362 Elevated Serum F-actin Levels May Reflect an Immune-Mediated Component to Liver Injury in HCV-Monoinfected and HCV/HIV-Coinfected Patients

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Background: Patients with autoimmune hepatitis (AIH) usually have elevated serum filamentous actin (F-actin) titers, although mildly increased levels have been described in other liver diseases, such as HCV infection. We have noted that rare chronic hepatitis patients with HCV, or HCV/HIV infection, have increased inflammatory disease activity and numerous plasma cells in the portal tracts of their liver biopsy samples, similar to patients with AIH. The purpose of this study was to evaluate the relationship, if any, between serum F-actin titers, hepatitis activity and the number of plasma cells present in liver biopsy samples from HCV and HCV/HIV infected patients.
Design: Liver biopsy samples from 18 HCV and 20 HCV/HIV infected patients, and 8 AIH controls, were evaluated for disease activity using a standardized 4-tier scoring system. Morphologic assessment was used to quantify the number of plasma cells (<5, 5-10, or >10) present in the most severely inflamed portal tracts (400x field), and confirmed with immunohistochemical stains (MUM-1 and CD138). For each case, serum F-actin levels (ELISA) were correlated with the pathologic features.
Results: Overall, elevated F-actin titers reflected increased disease activity ($p>0.05$) and were significantly correlated with the number of plasma cells present in biopsies from all groups (AIH: r=0.775, p=0.02; HCV: r=0.475, p=0.05; HCV/HIV: r=0.634, p<0.003).

Table 1. Clinical and Pathologic Features of Patients with Chronic Hepatitis

	AIH Patients	HCV Patients	HCV/HIV Patients
Mean Age (years)	50	51	47
Male/Female Ratio	1/7	16/2	11/9
Serum F-actin >30 ELISA Units	5 (63%)	5 (28%)	1 (5%)
Mean F-actin (ELISA Units)	31	22.9	16.3
Liver Biopsy Features			
Hepatitis Grade 0-2	6 (75%)	15 (79%)	17 (85%)
Hepatitis Grade 3-4	2 (25%)	3 (21%)	3 (15%)
<5 Plasma Cells	1 (12.5%)	3 (16.6%)	5 (25%)
5-10 Plasma Cells	3 (37.5%)	6 (33.3%)	6 (30%)
>10 Plasma Cells	4 (50%)	9 (50%)	9 (45%)

Conclusions: Elevated serum F-actin levels are frequently detected in patients with HCV-related chronic hepatitis and often reflect the presence of a plasma cell-rich inflammatory cell infiltrate, similar to that seen in AIH. It is possible that some HCV-infected patients develop an immune-mediated component to their hepatitis, which may be due to virally-mediated immune dysregulation, or a result of therapy.

1363 General Pathologist Inter and Intraobserver Variability in Knodell Scoring of Liver Biopsies from Patients with Hepatitis C

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Background: Accurate, reproducible interpretation of liver biopsies in patients with hepatitis C is crucial in monitoring disease progression therapeutic response. Our study evaluates the inter and intraobserver variation using the Knodell scoring system to independently assess portal and lobular inflammatory activity, necrosis, and fibrosis.
Design: Six pathologists independently assessed fifteen blinded liver biopsies. Interobserver correlation was assessed by Kappa (K) and slide-by-slide analysis. Slide-by-slide analysis was performed by determining the percentage of observers in agreement when variable levels of discordant scoring were allowed. Intraobserver variation was determined by comparing the initial and current interpretations by those pathologists who had initially evaluated ≥3 cases (4 observers). The inter and intraobserver correlations were then compared to those obtained in prior studies.
Results:

INTER AND INTRAOBSERVER CORRELATION

	UCSD Inter		Goldin†	Grombaek†	French†	UCSD Intra
	K	Wt K	Wt K	K	K	K
Portal	0.42	0.71				0.54
Lobular	0.53	0.72				0.57
Interface	0.37	0.74		0.34	0.15	0.63
Activity	0.30	0.48	0.47	0.39	0.43	0.62
Fibrosis	0.56	0.85	0.87	0.49	0.51	0.59
Grade	0.46	0.76				0.71

†Prior interobserver studies

Moderate correlations (K>0.4) were observed for all categories except interface necrosis and total activity, with the best correlation observed for fibrosis. Slide-by-slide analysis showed that 79.7% and 74.2% of observers agreed on fibrosis score and grade without variation. When one degree of discrepancy was allowed, >95% agreed. Conversely, 51.9% of observers agreed on total activity without discrepancy and four degrees of variation were required to attain >95% agreement. Intraobserver correlation was better than interobserver in all categories, with at least moderate correlation in all categories.
Conclusions: Our group of six general pathologists had similar or better interobserver correlations than were obtained in three prior studies. The best correlations and slide-by-slide agreement were attained for fibrosis (disease stage) and inflammatory grade, the two categories most utilized in evaluating disease progression and response to therapy. Intraobserver correlation was better than interobserver correlation in all categories. Our study validates the utility of the Knodell scoring system and highlights the need for adherence to scoring criteria in evaluating liver biopsies.

1364 Upregulated Micro RNA Expression in Pancreatic Ductal Adenocarcinoma, and Corresponding Cytology Specimens

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Background: Previous authors have described expression patterns of microRNAs (miR) to differentiate pancreatic ductal adenocarcinomas (PanCa) from normal pancreas (NPan) and chronic pancreatitis (ChrPan). Recently, newer techniques, like endoscopic ultrasound, have led to an increase in pancreatic cytology specimens. Our purpose was to determine if miR expression in pancreatic carcinoma cytology specimens, utilizing a set of four miR, correlates with miR expression in the subsequent pancreatic surgical specimen.
Design: H&E slides from 19 PanCa were reviewed. With Acturus Pixel II instrument, by LCM, ductal epithelium (DE) from 15µ paraffin sections was isolated using 7.5mm laser spot size, 70mW power and 750ms duration. From PanCa cases DE from tumor (T) and normal adjacent to tumor (Nt) was isolated. 10/19 PanCa cases had corresponding cytology (Cy) slides with positive diagnosis in 7 and 3 suspicious. Four miRs with previously reported upregulated expression in PanCa (miR155, 221, 181b, 181d) were analyzed using TaqMan MicroRNA Assays (Applied Biosystems, Foster city, CA), and RotorGene 3000 (Corbett Research, Sydney, Australia). Comparative C_t method was used for relative miR quantitation. The amount of target (tumor or cytology), normalized to an endogenous reference (RNU6B) and relative to a calibrator (normal tissue from the same patient, Nt) is given by $2^{-\Delta\Delta CT}$.
Results: Please refer to Table for results.

Expression of miRNA's in Pancreatic Ductal Adenocarcinoma

Fold Change	miRNA	No. Total=19	%age
UP 10x	miR155 (6), miR181d(2), miR181b(3), miR221(4)	15	75
UP 2-10x	miR155(3), miR181d(3), miR181b(7), miR221(3)	16	80
UP 1-2x	miR155(2), miR181d(1), miR181b(3), miR221(1)	7	35

Conclusions: 1. Cytology PanCa miR results correlate with the paired, subsequent surgical excision adenocarcinoma miR. 2. PanCa miR demonstrated a greater than 10 fold increase in expression of at least one of the miR in almost a third of all cases (6 of19) and there was at least a 1-2 fold increase in expression of at least one of the miR, compared to paired, histologically normal controls in most cases (15 of 19 cases). 3. The relatively low increase in miR expression, compared to the previous study, may reflect a more refined harvest technique (laser dissection) in our study. The previous study may have had a greater stromal dilutional effect in the normal and chronic pancreatitis cases than in the adenocarcinoma cases, allowing for greater separation between the populations.

1365 Cytokeratin 7 Hepatocyte Immunoreactivity in Chronic Allograft Rejection

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Background: Hepatocyte cytokeratin 7 (CK7) expression has been noted in a variety of ductopenic conditions (primary biliary cirrhosis, Alagille syndrome, progressive familial intrahepatic cholestasis) and is believed to reflect a metaplastic change in the setting of hepatocyte loss of contact with the biliary tree. We examined the pattern of hepatocyte CK7 expression in chronic allograft rejection, a ductopenic condition in which this phenomenon has not been systematically studied.
Design: Ten patients with the clinicopathologic diagnosis of chronic rejection were identified. Ten age, sex, and native disease matched patients served as controls. Biopsies obtained within 1 month of transplant (generally post-perfusion biopsies) and specimens diagnostic of chronic rejection in the "case" population and matched for time post-transplant in the "control" population were examined. The study pathologists independently reviewed H&E and CK7-stained sections, counting interlobular bile ducts (BD) and portal tracts (PT) and noting the morphologic pattern of injury in H&E sections and scoring hepatocyte CK7 immunoreactivity as follows: 0 none, 1+ rare, 2+ multifocal, predominantly periportal, 3+ as for 2+ but with extension into the lobule, 4+ diffuse. The study pathologists were blinded to clinical information and the

original histologic diagnosis. Mean BD/PT ratios and CK7 scores were calculated for each specimen. Specimens with < 5 portal tracts were excluded from further analysis. The Kruskal-Wallis test and Dunn's multiple comparison test were used to analyze group mean data.

Results:

Mean CK7 Scores and BD/PT Ratios in Cases and Controls

	Cases		Controls	
Time < 1 month	mean CK7	0.21	mean CK7	0.067
	mean BD/PT	1.1	mean BD/PT	1.2
	(n=8)		(n=5)	
Time of Diagnosis	mean CK7	1.5	mean CK7	0.19
	mean BD/PT	0.55	mean BD/PT	1.0
	(n=8)		(n=7)	

The p values for the Kruskal-Wallis tests of CK7 and BD/PT means were 0.0051 and 0.0007, respectively. Using Dunn's multiple comparison test, statistically significant differences were obtained when comparing mean CK7 scores and BD/PT ratios for the "cases at time of diagnosis" group with every other group ($p < 0.05$). All other comparisons were not statistically significant ($p > 0.05$).

Conclusions: Hepatocyte CK7 staining is present in chronic allograft rejection, and it would appear to reflect ductopenia, as there is no significant staining in matched controls. Further study is warranted to better characterize the relationship between CK7 staining and ductopenia and to assess its potential diagnostic utility.

1366 Morphoproteomic Profile of Signal Transduction Pathways of Convergence in Cholangiocarcinoma

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Background: Cholangiocarcinoma (CCA) is a rare disease with poor prognosis and short overall survival. Understanding pathways that contribute to its growth, integrity and metastatic potential might provide opportunities for targeted therapies. This study examines signal transduction pathways of convergence and cell cycle parameters in CCA, using a morphoproteomic approach. Specifically, we profile the state of activation of the mammalian target of rapamycin (mTOR), ras/Raf kinase/extracellular signal-regulated kinase (ERK) and nuclear factor (NF)-kappaB pathways and cell cycle associated protein analytes to include Ki-67 and S phase-associated protein kinase (Skp-2).

Design: Archival materials from 5 cases of CCA were studied. Immunohistochemical probes were utilized for the detection of the following: phosphorylated (p)-mTOR (Ser 2448) and its downstream effector, p-p70S6K (Thr 389); p-ERK 1 / 2 (Thr202/Tyr 204); p-NF-kappaBp65 (Ser 536); S100P, an upstream signaling ligand in the ERK pathway; Von Hippel-Lindau protein (pVHL), an antagonist in mTOR signaling; and Ki-67 for estimation of G1, S, G2 and M phases and Skp-2 for S phase. The signal and compartmentalization (plasmalemmal, cytoplasmic and/or nuclear) were assessed by bright-field microscopy (0-3+ scale); and the Ki-67 and Skp-2 nuclear signals represented as percentages. Non-neoplastic ducts (NND) were present in 4 cases.

Results: Moderate to strong (2+ and up to 3+) expressions of p-mTOR, p-p70S6K, p-ERK 1 / 2 and p-NF-kappaBp65 were evident in 4/5 (80%), 3/5 (60%), 3/5 (60%) and 1/5 (20%) of CCA, respectively. There was nuclear translocation for p-mTOR in 3/5 cases (a feature of activation) in contrast to plasmalemmal/cytoplasmic location in NND. Moreover, there was no expression of pVHL in CCA (0%) vs 2-3+ expression in all NND. Obversely, nuclear S100P was expressed in all CCA and absent from NND. Ki-67 and Skp-2 percentages ranged from 1.9 to 40% and 1.4 to 57%, respectively, and to some extent, paralleled the mTOR pathway expression.

Conclusions: Morphoproteomic analysis reveals a variable state of constitutive activation of pathways of convergence in CCA. mTOR pathway signaling (as evidenced by expression of the phosphorylation of mTOR and p70S6K on putative sites of activation, by nuclear translocation of p-mTOR and by G1 to S phase progression in the majority of cases of CCA) presents a focus for further study and a potential therapeutic target for individual patients with a similar morphoproteomic profile.

1367 Metastaitc Pancreatic Endocrine Tumors in the Liver Express KOC

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Background: Morphological determination of the origin of metastatic neuroendocrine tumors can be very difficult. Gastrointestinal tract and pancreas are two major origins of hepatic metastasis. Unfortunately, gastrointestinal carcinoid (GIC) and pancreatic endocrine tumor (PET) are morphologically similar. Although biomarkers, such as CDX2 and NESP55, have been used in the distinction, their application is limited by their low discriminating power. We recently reported expression of K homology domain containing protein overexpressed in cancer (KOC) in high-grade neuroendocrine tomors but not in carcinoids. We now tested if KOC could be used to separate GIC from PET in hepatic metastasis.

Design: 38 metastatic neuroendocrine/endocrine tumors of the liver (18 GICs, 16 PETs, and 4 of unknown primary) were immunostained with antibodies specific for CDX2, TTF-1, NESP55 and KOC. Nuclear (CDX2 and TTF-1), cytoplasmic (NESP55), and both cytoplasmic and membranous (KOC) stains were considered positive. The percentage of positively stained tumor cells was recorded and the staining intensity was graded as weak, moderate, or strong. A p value of <0.05, as determined by two-tailed Fisher exact test, was considered statistically significant.

Results: All 16 (100%) metastatic PETs were positively stained for KOC, with 12 (75%) showing a diffuse (>90% of tumor cells stained) and moderate to strong staining pattern. The remaining 4 exhibited a diffuse but weak positivity. Of 18 metastatic GICs, only 3 (17%) showed moderate staining detected in >50% of tumor cells ($p<0.01$; in comparison with PETs). Variable NESP55 staining was detected in 5 (31%) metastatic PETs but not in GICs. CDX2 was expressed in 17 (94%) GICs with moderate to strong nuclear staining detected in >50% of tumor cells, but CDX2 expression was also detected in 6 (38%) PETs. The 4 tumors with unknown primary were strongly and diffusely positive for CDX2, 2 of which also exhibited diffuse and moderate positivity for KOC. All tumors were negative for TTF-1.

Conclusions: KOC appears to have a more segregating power than CDX2 in distinguishing PETs from GICs. A combination of KOC and CDX2 immunostains provides more reliable tools in determining gastrointestinal or pancreatic origin of metastatic neuroendocrine/endocrine tumors in the liver.

1368 Circulating Microparticles as a Possible Diagnostic Marker in Liver Transplant Patients

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Background: Microparticles are small membrane vesicles released from the cell plasma membrane, particularly in cell stress, apoptosis and altered cellular viability. Increased numbers of circulating microparticles have been observed during cardiopulmonary bypass, unstable angina, lacunar infarcts and diabetes mellitus. Hepatocellular carcinoma (HCC) is a hypervascular neoplasm with high levels of apoptosis and necrosis. The aim of the current study was to investigate the levels of circulating microparticles of both hepatic and endothelial origin in patients with hepatitis C cirrhosis with and without HCC, as well as to determine if the numbers of circulating microparticles could be used as a novel marker for tumor growth and/or the clinical outcome in liver transplant patients.

Design: Using immunolabeling of microparticles of different origin and FACS-based enumeration of microparticles, the levels of circulating microparticles were studied in 16 patients, before and within two weeks after the transplant. Blood samples were collected before the surgery and on days 1, 7 and 14 following the transplant or partial hepatectomy. The number of circulating microparticles of different origin was enumerated in the blood samples using FACS analysis. Microparticles of different origins were identified by their immuno properties.

Results: The initial levels of circulating microparticles were increased in patients with HepC and HCC as compared to patients with HepC alone. Levels of endothelial and hepatic microparticles were increased in all liver transplant patients as compared to control patients. Levels of hepatic and endothelial circulating microparticles correlated directly with the tumor size. Levels of circulating microparticles were dynamically changing after the transplant, showing an initial increase with a subsequent decrease by the end of the second week after the surgery. In patients with a complicated clinical outcome, the levels of microparticles were continuously increasing after the surgery.

Conclusions: We demonstrated that the levels of circulating microparticles are well-correlated with both the tumor size and the clinical outcome. Prospectively, the levels of circulating microparticles may be used in clinical practice as a diagnostic marker of a malignant tumor as well as a marker of the liver functional status in liver transplant patients.

1369 Immunohistochemistry for PAX-2 Distinguishes Hepatocellular Carcinomas with Clear-Cell Morphology from Renal Cell Carcinomas

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Background: Immunohistochemistry for PAX-2, a transcription factor important to renal development, is positive in the majority of clear cell renal cell carcinomas (RCCs). The immunohistochemical profile of PAX-2 in hepatocellular carcinomas (HCCs) and hepatoblastomas (HBs) is not known. In adults, the differential diagnosis for hepatic neoplasms that display clear cell morphology, as can be commonly seen in HCCs, includes metastatic RCC. Moreover, the distinction can be especially challenging on small tissue samples or biopsies.

Design: We constructed tissue microarrays to investigate the immunostaining pattern of PAX-2 in HCCs and HBs. The tissue microarrays contained 1.5-mm cores of 150 HCCs and 84 HBs with a wide range of differentiation and morphologic subtypes. Of these cores, 13 HCCs (9%) showed clear cell morphology that could raise the possibility of a metastatic RCC. 13 HBs (15%) also contained significant clear cell change. We performed immunohistochemistry for PAX-2, EMA, and RCC antibody to evaluate the possibility of a renal metastasis, and HepPar1 and glypican-3 to evaluate hepatocellular differentiation; reticulin stains were also evaluated. Positive controls were performed simultaneously for each respective immunostain. The neoplasms were interpreted as positive for PAX-2, if they showed nuclear staining.

Results: All of the HCCs and HBs, including the HCCs with clear cell morphology similar to RCCs, did not show nuclear immunostaining for PAX-2. Furthermore, 147/150 HCCs and all of the HBs did not show reactivity for EMA. RCC antibody was negative in all. Immunohistochemical positivity for HepPar1 and/or glypican-3 was seen in 141 (94%) HCCs. Reticulin staining also helped to confirm these lesions as HCC. 20% of the HCCs and 12% of the HBs showed varying degrees of cytoplasmic or background staining for PAX-2, which is not considered true reactivity.

Conclusions: Nuclear immunoreactivity for PAX-2 was absent in HCCs and HBs with a wide range of differentiation and morphologic subtypes. PAX-2 may be useful in distinguishing between HCC with clear cell morphology and metastatic clear cell RCC, and may be especially useful in small biopsies, which are mimicked by the small size of tissue microarray cores used in this study.

1370 Hepatic Histopathologic Features of the American Lifestyle Induced Obesity Syndrome (ALIOS) Mouse Model

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Background: The ALIOS mouse model, a combination of sedentary lifestyle and common fast food diet (FFD), has been shown to successfully recapitulate many of the metabolic and clinical features of nonalcoholic fatty liver disease (NAFLD) without use of genetic aberrations, dietary extremes or toxic manipulations. The AIM is to describe the progression of hepatic histology.

Design: Male C57/BL6 mice were fed ad lib FFD (43% calories from fat that contains transfats) and gel water with 6g/kg/d high fructose corn syrup equivalent (ALIOS group) or standard chow and water (Control). ALIOS were sedentary by removal of cage racks. Grps of 10 were studied at 1,2,4,8,16,24 wks.

Results: All mice gained weight; ALIOS weighed 26% more than Controls at 24 wk. Liver weights of ALIOS nearly doubled by 16 wks; controls' were unchanged. At 1 wk, hepatic triglycerides in ALIOS were increased, but not Controls ($p < 0.01$); by 8 wk, serum ALT in ALIOS were increased ($p < 0.05$). In ALIOS, glucose tolerance was abnormal at 4 wk and fasting gluc was elevated at 6 wk. By 8 wk, mRNAs for TNFα and procollagen were increased 5.4 and 3-fold. Histol eval showed progressive increase in amount and size of fat droplets with time in ALIOS mice. At 1 wk 4 mice (40%) had barely discernable small droplets; at wk 2, 60% did and 4 had > 33% involvement. By wk 4, all ALIOS had diffuse small droplet steatosis and 2 had macrosteatosis. At 8 wk, all ALIOS had at least 33% macrosteatosis in zones 1-2; in most, subcapsular accentuation was noted; 30% also had true microsteatosis in zone 3. 80% of ALIOS livers at 16 wk had up to 99% steatosis with notable droplet size gradient from macro (zones 1,2) to micro (zone 3) maintained. 100% of 24 wk mice had 100% steatosis with maintenance of the zonal demarcations. Inflammation, rare but present, did not change with steatosis progression. Fibrosis was not observed by histochemical or IHC stains.

Conclusions: The ALIOS model rapidly develops impaired glucose tolerance and hepatic histologic features similar to human NAFLD. The pattern of liver involvement by 8 wks is reminiscent of pediatric (Type 2) NAFLD because of early zone 1 steatosis. The maintenance of macrosteatosis in zones 1 and 2, and microsteatosis only in zone 3, with ongoing accumulation indicates as yet unexplored zonation of triglyceride regulatory pathways. The subcapsular accentuation in many cases has implications in human studies.

1371 Portal Chronic Inflammation (CI) in Nonalcoholic Fatty Liver Disease (NAFLD): Correlations from the NASH Clinical Research Network (CRN)

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Background: Portal CI in NAFLD can occur with treatment, in pediatric NAFLD, and with concurrent disease. Aim: To evaluate correlates of portal CI in adult and pediatric liver biopsies (bxs) in the NASH CRN.

Design: The 10 pathologists of the Pathology Committee of the NASH CRN meet regularly to provide central, masked assessment of adult and pediatric liver bxs for the Database study and 2 treatment trials. Bxs are evaluated according to the published system (Hepatology 2005). Portal CI is scored "none", "mild", "more than mild". Clinical and bx data from 728 adult bxs and 205 pediatric bxs were studied. Ordinal logistic regression analysis was performed and models were adjusted for age at bx, race/ethnicity, and gender.

Results: In adult bxs, 17% had none, 62% had mild, and 24% had more than mild portal CI. The demographic and clinical features associated with increased portal CI were older age (OR=1.03, $p<0.001$), female gender (OR=1.45, $p=0.02$), higher BMI (OR=3.12 for BMI>35 kg/m2, $p<0.001$), and increased insulin values (OR=1.01, $p=0.01$). A history of antidiabetic (OR=2.38, $p<0.001$), anti-NAFLD (OR=1.72, $p=0.003$), and cardiovascular/hypertensive medications (OR=1.46, $p=0.02$) was also associated with increased portal CI. Associated histologic features were decreased steatosis (OR=1.60 for steatosis=5-33%, $p=0.01$), azonal location (OR=4.12, $p<0.001$), increased ballooning (OR=3.39 for "many" ballooned cells, $p<0.001$), advanced fibrosis (OR=15.2, $p<0.001$), and diagnosis of definite steatohepatitis (OR=2.7, $p<0.001$). In pediatric bxs, 10% had none, 76% had mild, and 14% had more than mild pCI. In contrast to adults, pediatric pts with portal CI were younger (OR=0.85, $p=0.02$), and less likely to use certain medications, such as antidiabetic (OR=0.19, $p=0.008$) and anti-NAFLD medications (OR=0.25, $p=0.02$). There was no association with gender, BMI, or insulin levels. The histologic features associated with portal CI in pediatric bxs were advanced fibrosis (OR=26.9, $p<0.001$) and diagnosis of definite steatohepatitis (OR=2.23, $p=0.002$).

Conclusions: Analysis of this large series of bxs from adult and pediatric patients with the histologic spectrum of NAFLD shows clinical and histological differences between the two. However, in common, in neither adult nor pediatric bxs was the degree of portal CI associated with race/ethnicity, raised ALT, presence of autoantibodies, or history of NAFLD-associated medications. Portal CI was also not associated with amount of lobular inflammation in either group.

1372 Mucinous Nonneoplastic Cyst of the Pancreas: Apomucin Profile Distinct This Entity from Intraductal Papillary Mucinous Neoplasm

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Background: Mucinous nonneoplastic cyst (MNC) of the pancreas is a newly described entity. By definition, MNCs are solitary and isolated unilocular or multilocular mucinous cystic lesions lined by single layer of cuboidal to columnar mucinous epithelium, with no ovarian type stroma and absence of communication with pancreatic duct. The origin and development of this entity is unclear. The identification of this lesion from other mucinous neoplasms of the pancreas such as branched duct IPMNs by morphology can be very challenging. In this study, we sought to examine MNC for: 1) apomucin characteristics by analyzing MUC1, MUC2 and MUC5AC expression immunohistochemically; and 2) to perform clonality analysis for confirming its non-neoplastic nature.

Design: 436 resected pancreatic tumorous lesions between 2002 and 2007 in our institution were reviewed. Apomucin immunostains (MUC1, MUC2 and MUC5AC) were performed on MNCs and IPMNs using standard immunopeoxidase method. Clonality assay of NMC was performed by PCR-based clonality analysis with the HUMARA gene for micro-dissected mucinous epithelia cells from paraffin sections.

Results: 14 (3.2%) MNCs were identified in our series. They included 3 males, 11 females with a median age of 60 years. 8 cases (57%) occurred in pancreatic head, 2 (14%) in neck and 4 (29%) in tail. The size of cysts ranged from 0.5-3.5 cm in greatest dimension. The majority of lesions (11/14, 78%) were associated or adjacent to acinar-ductal mucinous metaplasia. Apomucin immunostains of MNCs showed: MUC1 expressed in 4 cases (29%); MUC5AC in 9 cases (65%); MUC2 was negative in all cases. This apomucin profile was very similar to adjacent acinar-ductal mucinous metaplasia. By comparing to IPMNs (n=5), all of the IPMNs exhibited MUC2 positivity (most showing diffuse pattern expression) and MUC5AC positivity, while none were MUC1 positive. Furthermore, the clonality assay with the HUMARA gene revealed that the NMCs were polyclonal origin.

Conclusions: Our results indicate that MNCs are rare cystic lesions in the pancreas, commonly associated with adjacent acinar-ductal mucinous metaplasia and share similar mucin profile, suggesting possible originated from acinar-ductal mucinous metaplasia. The polyclonal nature demonstrated by clonality analysis support our hypothesis that theses lesions are non-neoplastic. Furthermore, MUC2 may serve as unique biomarker to differentiate NMCs from branched duct IPMNs.

1373 Microvessel Density in the Malignant Progression of Hepatocellular Carcinoma Arising from Cirrhotic Liver in Comparison with Those from Non-Cirrhotic Liver

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Background: Angiogenesis is essential to the survival, growth, invasion, and metastasis of various human solid tumors. We studied the association of microvessel density (MVD) with the malignant progression of hepatocellular carcinoma (HCC) from cirrhotic liver, macroregenerative nodules (MRN) to dysplastic nodules (DN) and from well to poorly differentiated HCC. We also compared the MVD between HCC arising from cirrhotic (HCC-C) and non-cirrhotic (HCC-NC) livers.

Design: Tissue microarrays were constructed with 17 normal livers, 16 cirrhotic livers, 36 MRN, 12 DN, and 56 HCC (6 well-differentiated, 42 moderately-differentiated, and 8 poorly-differentiated) of which 39 were HCC-C and 17 were HCC-NC. It was stained immunohistochemically with antibodies against the antigen CD34. The MVD was determined by the measurement of the area of CD34 positive sinusoidal endothelial cells using the Image Pro Plus software®.

Results: There was a trend of increased MVD in cirrhotic liver compared to normal liver ($p = 0.057$). MRN and DN both showed a statistically significant higher level of MVD than normal liver ($p = 0.005$ and 0.002, respectively). There was no statistical difference in MVD between MRN and DN ($p = 0.40$). In comparison with normal liver, cirrhotic liver, MRN and DN, HCC showed a higher MVD ($p <0.001$, <0.001, <0.001, and $p = 0.006$, respectively). There was no statistical difference in MVD among different grades of HCC. Although both HCC-C and HCC-NC showed significant increase in MVD compared to their background liver ($p = 0.007$ and <0.001, respectively), there was no statistical difference in MVD between HCC-C and HCC-NC ($p = 0.12$).

Conclusions: In HCC-C, sinusoidal capillarization seems to parallel the malignant progression from cirrhosis to precursor lesions and to HCC. However, without these precursor lesions, sinusoidal capillarization also occurs in HCC-NC. As a marker of malignancy, sinusoidal capillarization appears more specific in HCC-NC than in HCC-C.

1374 Diabetic Hepatosclerosis: A 10 Year Autopsy Series

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Background: Diabetic hepatosclerosis (DHS) was described in clinically-indicated liver biopsies (bxs) of 12 patients (pts); all had other severe manifestations of Type 1 Diabetes Mellitus (T1DM). DHS may be a microangiopathic complication of T1DM (Arch Pathol Lab Med 2006). Aim: To evaluate prevalence and compare the histologic and clinical findings of DHS in an autopsy population.

Design: Liver slides from all autopsied diabetics that included abdominal organs from 1997-2007 were reviewed by H and E, trichrome and PASd. Cases of NAFLD/NASH and cardiac sclerosis were excluded to avoid potential over-diagnosis. Other exclusions were autolysis and cirrhosis. DHS was defined as dense trichrome positive perisinusoidal fibrosis. Additional features were perivenular fibrosis, hepatic artery (HA) thickening, PASd positive HA deposits or concentric layering.

Results: 976 autopsies yielded 254 with DM; 159 had evaluable liver slides. 19 (12%) had DHS. In contrast to the 12 pts of Harrison et al, our pts were M>F (14:5) (Harrison et al, M<F 4:8), older (mean age 56.4 y, range 37-81, compared to mean 45.8 y, range 28-68y), IDDM reported in 53% (3 not reported) compared with 83%; and alkaline phosphatase (alk phos) raised in 32% (2 not reported) compared with 83%. Both studies had 2 pts with HCV. Similar to Harrison et al were: DM renal complications (89% compared with 83%); retinopathy was less common in our study (16% v 42%). Macrovascular complications of DM were found in 100% of pts including recent or remote MI, coronary artery disease, CVA, and/or amputations. Histologic features included patchy distribution of DHS 42%; zone 3 predominant in only 37%; the remainder were zone 3 + 2: 47%, azonal: 16%. Eccentric perivenluar fibrosis was noted widely in 84%. Portal tract small HA branch thickening by trichrome was seen

in 63%; most (58%) was due to PASd amorphous deposits within the vessel wall. Portal lipogranulomas were prominent in 58% of cases. The PASd stain did not accentuate the psf or perivenular fibrosis to the same extent as the vessel deposits and/or concentric layering.
Conclusions: The report of Harrison et al was in pts who had liver bx for elevated liver tests. Our series is an autopsy population of DM (excluding cirrhosis or cardiac sclerosis). We examined 159 livers and documented DHS in 12%. There are interesting contrasts with the initial report of DHS in that the autopsy pts were older, had a male predominance and has less liver-related clinical manifestations (e.g. raised alk phos). However, similarities include severe renal disease, and changes in portal tract HAs. The rarity of DHS is confirmed.

1375 Mist1 Expression in Acinar Carcinomas and Intraductal Oncocytic Papillary Neoplasms of the Pancreas

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Background: Mist1 is a basic helix-loop-helix transcription factor that is expressed during pancreatic development and required for proper pancreatic acinar organization. In the adult pancreas, Mist1 expression is restricted to acinar cells. We evaluated the expression of Mist1 in a panel of human pancreatic neoplasms including acinar cell carcinomas (ACC), mixed neoplasms with an acinar cell differentiation (MCA), intraductal oncocytic papillary neoplasms (IOPN), and pancreatoblastomas.
Design: 58 pancreatic neoplasms were studied including 22 ACCs, 20 IOPNs, 12 MCAs, and 4 pancreatoblastomas. Immunohistochemical (IHC) labeling was performed using an affinity-purified rabbit antibody (Ab) specific to Mist1 protein. Sections were reviewed by at least 3 independent observers. Nuclear labeling of greater than 5% of the neoplastic cells was classified as positive.
Results: Mist1 was expressed in normal acinar cells, but not in normal ductal epithelial cells or islet cells. 7 of 22 (32%) ACC and 4 of 12 (33%) MCA expressed Mist1. In one positive MCA, Mist1 expression was observed in neoplastic cells with both acinar cell and ductal differentiation. Surprisingly, 11 of 20 (55%) IOPNs expressed Mist1. Positive nuclear labeling of IOPNs was observed in neoplastic cells with prominent granular eosinophilic cytoplasm as well as those with intracytoplasmic mucin vacuoles. The pancreatoblastomas (0/4) did not label for Mist1.
Conclusions: Mist1 is a specific IHC marker for acinar cells in the adult pancreas. Mist1 is expressed in more than half of IOPNs, suggesting a novel pathway of acinar cell differentiation in these unique neoplasms of the pancreas. Paradoxically, Mist1 is only expressed in about one-third of ACC and MCA. This latter finding suggests that Mist1 expression is lost in the carcinogenesis of acinar cell malignancies. The low sensitivity of Mist1 for true acinar neoplasms limits any potential dignostic utility of the Ab.

1376 Epstein-Barr Virus (EBV) Is Not Responsible for the Inflammatory Pseudotumor like Morphology and Venulitis Associated with Lymphoplasmacytic Sclerosing Pancreatitis

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Background: Lymphoplasmacytic sclerosing pancreatitis (LPSP) is a distinct form of chronic pancreatitis, histologically characterized by mixed lymphoplasmacytic inflammation centered around the pancreatic ducts and venulitis. Abundant IgG4-expressing plasma cells are often present. In many cases there is prominent sclerosis and expansion of periductal tissue which can be so prominent as to resemble inflammatory pseudotumor (IP). Although LPSP has been associated with other autoimmune diseases, the definitive etiology of the disease is still elusive. Since EBV has previously been reported to play a role in the development of inflammatory pseudotumor and EBV can cause venulitis, we studied a large series of LPSP cases for EBV RNA.
Design: 21 cases of LPSP were studied from 14 men and 7 women aged 40-78 years (mean 63). Partial or total pancreatectomy specimens were examined in each case. H&E stained sections were reviewed and representative sections were selected for EBV early RNA (EBER) *in situ* hybridization. The selection criteria included a presence of prominent spindle cell proliferation resembling inflammatory pseudotumor and/or the presence of prominent perivenulitis/frank venulitis. Any definitive nuclear labeling was considered positive.
Results: All 21 cases demonstrated areas of prominent spindled fibrous cell proliferation. Positive and negative controls for EBER labeled appropriately. All 21 cases of LPSP were EBER negative. The spindle cells, ductal epithelial cells, acinar cells, endothelial cells, lymphocytes and plasma cells did not label.
Conclusions: LPSP frequently demonstrates a focal spindle cell proliferation and venulitis. Using EBER *in situ* hybridization, we were not able to demonstrate EBV in a large series of cases of LPSP.

1377 Expression of p16 in Gallbladder Adenocarcinoma

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Background: Adenocarcinoma of the gallbladder is a highly malignant neoplasm. p16 is a tumor suppressor gene protein, which is a cyclin-dependent kinase inhibitor that regulates the G1-S phase of the cell cycle. The purpose of this study was to investigate the expression of p16 in precancerous lesions of gallbladder and gallbladder adenocarcinomas and to examine the relationship between this expression and clinicopathologic parameters.
Design: Formalin-fixed, paraffin-embedded tissue sections from 20 cases of normal gallbladder, 20 cases of chronic cholecystitis, 20 cases of gallbladder adenoma, 20 cases of gallbladder dysplasia, and 58 cases of gallbladder adenocarcinoma were examined. The expression of p16 was evaluated by immunohistochemical analysis.
Results: In normal gallbladder, expression of p16 expression was not found. In chronic cholecystitis, expression of p16 expression was not found. In gallbladder adenoma, expression of p16 was found in 20% (4/20). In gallbladder dysplasia, expression of p16 was present in 45.0% (9/20). In gallbladder adenocarcinoma, expression of p16 was found in 27.6% (16/58). Expression of p16 correlated significantly with histologic grade ($P<0.05$). Expression of p16 was significantly higher in gallbladder adenocarcinoma than that in normal gallbladder and chronic cholecystitis, respectively ($P<0.01$). There was no correlation between gallbladder adenoma, dysplasia and adenocarcinoma in p16 expression. p16 expression did not correlate significantly with age, gender, tumor size, gross type, location, vascular invasion, lymph node metastasis, and TNM stage, respectively.
Conclusions: p16 plays a role in the early carcinogenesis of the gallbladder adenocarcinoma. p16 expression may be correlated with histologic grading of the gallbladder adenocarcinoma.

1378 Phosphorylated c-Met Is Preferrentially Located in the Nucleus of Hepatoblastomas

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Background: c-Met, a receptor tyrosine kinase that undergoes autophosphorylation upon ligand-binding activation, plays an important role in tumorigenesis and is a current chemotherapeutic target. c-Met has been found to be mutated and over-expressed in a variety of tumors, including hepatablastomas (HBL). In order to understand the role c-Met plays in HBL, the first large-scale analysis of total c-Met and triple-phosphorylated c-Met (p-cMet) is presented.
Design: We created a Tissue Microarray (TMA) using paraffin-embedded tissue of 52 HBL and 33 adjacent normal liver from the SIOPEL (The International Childhood Liver Tumour Strategy Group) 3 standard risk trial. A TMA with 50 hepatocellular carcinomas (HCC) was created at the University of Chicago. Using immunohistochemical techniques, the TMAs were analyzed for the cytoplasmic and nuclear presence of total c-Met and p-cMet.
Results: p-cMet is present in 82.7% and 75.8% of HBL and normal adjacent liver, respectively. However, in the HBL, nuclear staining is present in 76.9% and cytoplasmic staining is present in 38.5% ($p<0.01$) of the cases. In normal tissue, nuclear and cytoplasmic staining are observed in 33.3% and 63.3%, respectively ($p=0.077$). These data are summarized below. All normal liver with nuclear staining have nuclear staining in the tumor correlate. Four out of 5 pure embryonal or predominantly embryonal HBL are negative for p-cMet. In HCC, p-cMet is present only in the cytoplasm. Total c-Met is present only in the cytoplasm of all tissue analyzed.
Conclusions: All of the normal liver with nuclear p-cMet is present in a background of HBL with nuclear p-cMet. These normal liver tissues may represent premalignant lesions. More importantly, a significant portion of HBL have nuclear versus cytoplasmic p-cMet staining, whereas all HCC have only cytoplasmic p-cMet. These data suggest a different biological role for p-cMet in HBL that may be utilized as a future therapeutic target. In light of the observation that embryonal HBL, a more undifferentiated tumor, is negative for p-cMet, HBL are an attractive model to study the biology of p-cMet. Future studies include correlation with clinical and survival parameters.

	Phospho-c-Met			
	Nuclear	Cytoplasm	Nuclear only	Cytoplasm only
Hepatoblastoma	76.9% (40)	38.5% (20)	44% (23)	5.8% (3)
Normal Liver	33.3% (11)	63.3% (21)	12.1% (4)	36.4% (12)

1379 Wilson's Disease: Histopathological Correlations with Treatment

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Background: Wilson's disease (WD) is an autosomal recessive inherited disorder of copper metabolism, resulting in abnormal copper deposition in various tissues, and producing hepatic, neurologic and/or psychiatric disease. Liver involvement is variable, ranging from enzyme elevation to cirrhosis. We report a cohort of WD patients treated with zinc and/or penicillamine who underwent multiple follow-up liver biopsies.
Design: Twelve patients with WD from the Division of Gastroenterology and Hepatology, Padova University Hospital, Italy, were followed from 1981 to 2006. Demographic, clinical, and laboratory data were gathered, and all patients underwent an initial biopsy and at least one repeated biopsy. Slides of formalin-fixed, paraffin-embedded tissue were stained with H&E, Perl's for iron, Van Gieson, and periodic acid Schiff with diastase. The biopsies were graded on three main parameters (inflammation, steatosis, fibrosis). Features such as hepatocellular injury and glycogenated nuclei were noted.
Results: Time to repeat biopsy ranged from 2 to 12 years. Three patients (group 1) showed histologic improvement: all with decreased steatosis and one with improvement of all three parameters. Five patients (group 2) had more severe inflammation and/or fibrosis. Four patients (group 3) showed no significant change on repeat biopsy, although two in this group had no histologic abnormalities on initial and repeat biopsies. Patients in groups 1 and 3 showed improved or stable transaminases, the initial value having been somewhat higher in group 1. Patients in group 2 showed either enzyme elevation or reduction. Hepatic copper concentration was initially greater in the group (3) that did not show signfiicant change. It both increased and decreased with treatment in both groups 1 and 2, while levels in group 3 decreased progressively.
Conclusions: Previous work has shown overall histologic improvement and reduction in hepatic copper with zinc therapy. In our study, all three patients who improved showed decreased steatosis, and one had less fibrosis, whereas patients who progressed demonstrated worsening inflammation and/or fibrosis. Serum aminotransferases tended

to correlate with histology in the group that showed improvement and those who remained stable. Urinary and hepatic copper concentrations were variable in all three groups, and did not correlate with histology or treatment with penicillamine or zinc.

1380 Common Variable Immunodeficiency Involving the Liver

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Background: Common Variable Immunodeficiency (CVID) is the second most common primary immunodeficiency disease. Histologically, a paucity of plasma cells in gut mucosal biopsies can aid in the diagnosis. However, in CVID there is a significant prevalence of chronic inflammatory diseases that affects a variety of organ systems beyond the GI tract, sometimes with granulomatous inflammation. This chronic inflammation is of uncertain pathogenesis, but is typically considered to be autoimmune in nature. Elevated liver enzymes can occur in patients with CVID, leading to liver biopsy, but the histological features of CVID in the liver have not been well described. The goal of this study was to further characterize the histological findings in liver biopsies of patients with CVID.

Design: All liver biopsies in our files were identified in patients with CVID. The CVID patients had no known viral hepatitis or other known active systemic infections. Using the Ishak scoring system, liver biopsies were evaluated for the extent of inflammatory activity and degree of fibrosis. The presence of granulomas, plasma cells, and fatty change was also noted.

Results: Liver biopsies were performed to evaluate the etiology of elevated liver enzymes. 11 liver biopsies (9 needle; 2 wedge) from 7 patients (3 female; 4 male) with CVID were identified (age range 2-67; mean 26). Three individuals (43%) showed scattered portal and lobular granulomas. In one of these individuals, the granulomas were present on a repeat biopsy. No plasma cells were identified in any of the cases. Overall, the biopsies showed a mild non-specific hepatitis with an average Ishak activity index of 4 (range 1-8). Interestingly, the inflammation tended to be predominantly lobular with the average lobular inflammation score of 2 (range 1-3), while portal inflammation had an average score of 1.4 (range 0-3). Interface activity ranged from none to focally present and overall tended to be mild. Mild portal fibrosis was seen in 7 cases, while 1 had moderate portal fibrosis, and 3 had no fibrosis. Mild macrovesicular steatosis was identified in 2 patients.

Conclusions: CVID frequently shows granulomas in the liver along with a mild lobular hepatitis and a lesser amount of portal inflammation and interface activity. While no cases with advanced fibrosis were found, the chronic CVID hepatitis can lead to mild fibrosis as demonstrated by most cases. While the etiology of the CVID hepatitis remains unclear, knowing the histological pattern of CVID is important when evaluating liver biopsies in this unique patient population.

1381 IgM and IgG Immunostaining Differentiates Primary Biliary Cirrhosis from Autoimmune Hepatitis

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Background: Primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH) are autoimmune diseases that affect the liver. In both PBC and AIH, patients show elevated serum gamma globulins, but the pattern is different, with IgM elevations in those with PBC and IgG elevations in those with AIH. The elevated serum gamma globulins in these conditions correspond with increased plasma cells on biopsy. PBC and AIH can show considerable histological overlap, with abundant plasma cells present in both conditions. We investigated whether immunophenotyping of plasma cells in liver biopsies would be helpful to differentiate PBC and AIH histologically.

Design: Established cases of PBC and AIH were identified from the pathology database (1987 through 2007) in which immunohistochemical stains for IgM and IgG had been performed at the time of clinical sign-out. The degree of inflammation and fibrosis was semi-quantitated using the Ishak scoring system. The presence of granulomatous inflammation was also noted. Immunohistochemical stains for IgM and IgG were blindly reviewed and the cases divided into two groups: 1) cases in which the number of IgG positive plasma cells exceeded the IgM positive plasma cells, and 2) those in which the IgM positive plasma cells were present in numbers greater than or equal to IgG positive plasma cells.

Results: 15 patients (12 female; 3 male) with PBC were identified (age range 38-71; mean 55). 40 patients (30 female; 10 male) with AIH were identified (age range 20-87; mean 48). PBC cases had an average activity index of 4 (range 1-7); while AIH cases had an average activity index of 8 (range 3-11). For PBC, 7/15 cases had no fibrosis, 5/15 cases had portal fibrosis (stages 1-2), and 3/15 cases had advanced fibrosis (stages 3-6). For AIH, fibrosis was absent (6/40), portal (8/40), and advanced (26/40). Over half (53%) of PBC cases showed granulomatous inflammation. PBC was strongly correlated with a predominance of IgM positive plasma cells, while AIH strongly correlated with an excess of IgG positive plasma cells (Table 1; Fisher's exact test, $p < 0.0001$). These findings held true regardless of the extent of inflammatory activity or degree of fibrosis.

Table 1: Immunohistochemical staining of plasma cells on liver needle biopsies

	IgM ≥ IgG	IgG > IgM
PBC (n=15)	13	2
AIH (n=40)	0	40

Conclusions: Immunohistochemical staining for IgM and IgG is helpful in differentiating PBC from AIH. The pattern of immunohistochemical staining is conserved, regardless of the extent of inflammation or degree of fibrosis.

1382 Factors Affecting Disease Progression in Chronic Hepatitis C: A Study of 387 Liver Biopsies from 128 Patients

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Background: The evaluation of liver biopsies has become increasingly important for assessing progression of disease and tailoring treatment for patients with chronic hepatitis C. Assessing other factors known to affect disease progression, such as viral genotype, is also vital to patient care. In this study we evaluated other elements, such as degree of steatosis, age at first biopsy, and degree of siderosis that may contribute to progression and severity of disease.

Design: We reviewed liver biopsies and medical records from patients with chronic hepatitis C biopsied between 1982 and 2007. Patients with more than one biopsy were selected in order to assess progression of disease. 387 adequate (> 4 portal tracts, >1 cm) liver biopsies from 128 patients were evaluated using H&E, trichrome, and Prussian Blue stains by two independent teams. Steatosis and steatohepatitis were evaluated using the Modified Brunt criteria. The Scheuer classification was used to assess the grade and stage of hepatitis. The degree of siderosis was graded on a scale of 1 to 4. Portal tracts were counted to assess biopsy adequacy. Records were reviewed to identify the patient's viral genotype, initial viral load, serum chemistries, and the start date for treatment, if applicable.

Results: Average age at first biopsy was 42.5 ± 7.6 years, with 64% males and 36% females. 78% of patients were genotype 1, with 19% being genotypes 2 or 3. Median time to progression was 60 months. 33% of patients in both the treated and untreated group had progression of disease. Higher stage at first biopsy was associated with higher steatosis ($r=.20$, $p=.03$), presence of steatohepatitis ($p<.01$), higher grade ($r=.53$, $p<.01$), fewer platelets ($r=-.23$, $p=.01$), and higher ALT ($r=.27$, $p<.01$

Conclusions: Both stage and grade at the first biopsy were predictive of progression. Those at grade 1 during the first biopsy were more than 5.5 times more likely to progress to the next stage (OR=5.63, $p=.04$) compared to those in Grades 2 & 3. Those in either stage 0 or stage 1 were more likely to progress to the next stage compared to those in stage 2 and 3. Those with genotype 1 were also more likely to progress to the next stage (OR=2.6, $p=.08$)). A higher hazard of disease progression was shown in those who were at stages 0 or 1 at baseline, those who were at grade 1 at baseline, and in those over 40 years of age at the first biopsy. Those with higher albumin levels had a lower hazard of disease progression.

1383 Autoimmune Pancreatitis Related Sclerosing Cholangitis, Primary Sclerosing Cholangitis and Steroid Responsive PSC: A Comparative Study of 27 Cases

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Background: Autoimmune pancreatitis related sclerosing cholangitis (AIP-SC) is a steroid responsive disease that mimics primary sclerosing cholangitis (PSC) clinically and radiologically. While classic PSC is resistant to steroids, a subset of cases have been reported to be steroid responsive (PSC-SR). The aim of this study was to perform a morphological analysis of AIP-SC, PSC, and PSC-SR livers, in an attempt to identify features that would distinguish AIP-SC from PSC, and to explore the pathology of PSC-SR.

Design: The study group consisted of livers from 9 AIP, 16 PSC, and 2 PSC-SR cases. All AIP-SC cases showed pancreatic involvement, and all 5 pancreatectomy specimens presented characteristic histopathological features of AIP. In the other 4 cases the diagnosis of AIP was made on clinical, radiological and immunological grounds. PSC cases were defined by the presence of a characteristic ERCP appearance. Clinical, pathological, radiological, and follow-up data was recorded on all cases. Portal and periportal inflammation was graded according to Ishak's guidelines.

Results: The AIP patients were older than individuals with PSC. On cholangiography, 5 of the AIP cases showed exclusively distal bile duct strictures, 3 demonstrated multiple intrahepatic biliary strictures, and one case showed a normal cholagiography. Both PSC-SR cases showed a PSC-like cholangiographic appearance. AIP livers showed higher portal inflammatory scores ($p=0.006$), and increased numbers of plasma cells ($p=0.001$). Microscopic portal based inflammatory pseudotumors composed of fibroblasts, plasma cells, lymphocytes and eosinophils were exclusively seen in 3 AIP and PSC-SR livers. The PSC-SR cases showed a morphological overlap with the AIP livers.

Conclusions: Portal based inflammatory pseudotumor distinguish AIP-SC from PSC livers. The portal tracts of AIP-SC livers are significantly more inflamed with a significantly larger plasma cell population than PSC livers. Further immunological studies are required to clarify the relationship of AIP-SC with PSC-SR.

	Mean age	Male:Female	Pseudotumor	Plasma cells 2+	Eosinophils	Phlebitis
AIP-SC	69	9:1	4 (44%)	5 (56%)	6 (67%)	5 (56%)
PSC	41	13:3	0	0	4 (24%)	2 (13%)
PSC-SR	41	2:0	1 (50%)	1 (50%)	1 (50%)	0

1384 A Novel Hepatitic/Ductular Lesion in HIV-Hepatitis C (HCV) Coinfected Liver Transplant Patients

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Background: We present a unique histologic lesion with features of both hepatitis and ductular changes that has never been described in the literature, occurring in patients with HIV and HCV who undergo orthotopic liver transplantation (OLT).

Design: 40 patients with HIV and either HCV or HBV underwent OLT*. Post-transplant liver biopsies were evaluated semi-quantitatively for inflammation, hepatocyte swelling, apoptosis, ductular reaction, cholestasis, fibrosis, and fat. Biopsy findings were correlated with clinical features (biliary obstruction, viral titers, CD4 count, and antiretroviral and immunosuppressant drug regimens) using Fisher's exact tests.

Results: 19 of 40 patients (48%) developed a combination of histologic findings consisting of prominent periportal ductular reaction with pericholangitis, associated with hepatitic features including mononuclear portal and lobular infiltrates, apoptosis of hepatocytes, and variable hepatocyte swelling. Only HCV coinfected patients (n=36) were affected, and 75% of patients on either efavirenz (7/9) or nevirapine (2/3) within 14 days post-transplant developed the lesion (p=0.04). Biliary obstruction was a clinical consideration in 10 patients and confirmed in 7, but no statistical significance was found for this factor (p=0.06), or for other antiretroviral drugs, age, sex, race, HIV or HCV titers, or CD4 counts. A trend for significance for cyclosporin use was seen (p=0.07). Median time to development of the lesion was 45 days post-transplant (range 3-296). The ductular component resolved after a median 55 days (range 12-659) in 8 patients for whom follow-up was available. Hepatitic changes persisted in 7 of these 8 patients without progression to cirrhosis or graft loss. Resolution was associated with HCV clearance in one case, and with cessation of nevirapine, then efavirenz, in one case.
Conclusions: Patients coinfected with HIV and HCV often develop a unique hepatitic lesion with early or prominent ductular reaction. This may represent an unusual immune response to HCV, independent of viral titer, perhaps altered by nevirapine or efavirenz use early after OLT. It is important to distinguish this lesion from biliary obstruction, rejection, or aggressive HCV. A larger sample is necessary to fully examine these findings in multivariate analyses. *Supported by the Solid Organ Transplantation in HIV: Multi-Site Study (AI052748) funded by the National Institute of Allergy and Infectious Diseases.

1385 Analysis of Nucleic Acid Index in Hepatocellular Carcinoma, Dysplastic Nodules, Regenerative Macronodules and Cirrhotic Liver

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Background: Hepatocellular carcinoma (HCC) is associated with 'precursor lesions' in the context of liver cirrhosis (LC): dysplastic nodules (DN), small (SCC) and large cell change (LCC). Differential diagnosis between a DN, regenerative macronodules (RMN) and well-differentiated HCC may be challenging and subjective. Nucleic acid index (NAI) assesses nucleic acids derangements in histological sections at the level of the individual cell and its environment, using confocal laser scanning microscopy (CLSM). It has been demonstrated that lower NAI values in melanocytic lesions appear to correlate with malignant potential. Our hypothesis is that NAI may be a useful adjunct to traditional light microscopy diagnosis, especially in borderline lesions.
Design: Thirty-six cirrhotic livers from autopsy cases were retrospectively studied. Formalin fixed, paraffin embedded tissue representing RMN, SCC, LCC, DN, HCC and LC surrounding parenchyma were included. LC samples were grouped according to clinical-pathological parameters in chronic hepatitis (CHP) or steatohepatitis (SHP) patterns; cirrhosis with or without HCC were compared. The sections were stained with acridine orange, a fluorescent stain for DNA and RNA and CLSM was performed. Average fluorescent intensities were measured in nuclei and the surrounding cytoplasm using Image-Pro Plus (MediaCybernetics Inc., Silver Spring, USA) software (v5.1.2). Three to eight high power fields were analyzed for each sample, and 3 to 6 nuclei/cytoplasm regions were measured in each field to determine the mean and standard deviation. NAI reflects the concentration of DNA relative to RNA.
Results: NAI in LC with (n=11) and without (n=23) HCC was respectively 1.57 ± 0.17 and 1.62 ± 0.68 (ns). LC in CHP (n=21) demonstrated a higher NAI than in SHP (n=10):1.86 ± 0.63 and 1.15 ± 0.41 respectively, $p<0.05$, t test. Average NAI in RMN (n=8), SCC (n=6), LCC (n=4), low grade DN (n=2), high grade DN (n=1) and HCC (n=4) were respectively 1.92 ± 0.13; 1.91 ± 0.10; 1.92 ± 0.46; 0.71 ± 0.07; 1.54 ± 0.18 e 2.01 ± 1.42. DN and well differentiated HCC trended to have lower NAI, although not reaching statistical significance ($p=0.07$).
Conclusions: The NAI/CLSM assessment may contribute to a better understanding of nodular lesions in cirrhosis and may serve as and adjunct to morphology improving objective diagnosis, especially in differentiation of cirrhotic patterns and regenerative nodules from dysplastic nodules and low grade HCC.

1386 Marked Iron Accumulation in Liver Explants: A Risk Factor for Cardiac Failure (CF) Even in the Absence of Major Gene Defects of Hereditary Hemochromatosis (HH)

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Background: Patients with HH are recognized to have increased risk for morbidity and mortality due to iron deposition in other organ systems following liver transplantation (OLT). We now report a unique set of patients with marked iron deposition in the native livers who develop CF following OLT and have excess iron deposits in the heart and other organs despite the absence of major gene defects of HH.
Design: Patients with marked iron overload in the liver explant and with subsequent CF were identified; patients with a pre-transplant diagnosis of HH were excluded. Medical records were reviewed for clinical and laboratory findings, and outcomes. To exclude patients with undiagnosed HH, DNA was extracted from paraffin blocks of the liver explant, amplified, and directly sequenced in the forward and reverse direction for the two major gene defects of HH: C282Y and H63D. Explants were also evaluated with iron stains, and iron graded on a 0-4 scale. Other pathology samples were examined for iron when available.
Results: Four patients, transplanted for ETOH and cryptogenic cirrhosis, age range 35 to 62 yrs, with cirrhosis, iron overload, and CF were identified. Molecular studies confirmed 3 of 4 were negative for C282Y and H63D; one was heterozygote for C282Y. Explants showed marked iron accumulation with >90% of the iron in hepatocytes: 4+ at the periphery of the nodules, predominantly 0-1+ in center of nodules. The heterozygote for C282Y+ had more diffuse iron deposits throughout the nodules typical of HH. In the HH- patients, 2 patients had cardiac evaluations prior to OLT with echocardiograms demonstrating left ventricular ejection fractions (LVEF) that ranged from 65 to75%, but within 3 months post OLT, LVEF ranged from < 20% to 35%; 1 patient had cardiac transplant; 1 died due to sepsis, was autopsied, and showed iron in the pancreas and thyroid. Cardiac tissue obtained from autopsy (1), endocardial biospy (2), or cardiac transplant (1) showed interstitial fibrosis and myocyte hypertrophy and iron deposits.
Conclusions: Patients with cirrhosis and hepatic iron, but no major gene defect of HH, may develop CF after OLT and may also have iron in other organs. Proper evaluation of the pre-transplant or explanted liver with attention to quantity and pattern of iron deposits could identifiy these patients to avoid post OLT cardiac complications and/or death. The etiology of the systemic iron overload remains to be discovered.

1387 Characteristic Clinicopathological Phenotypes of the Types of Intraductal Papillary-Mucinous Neoplasm of the Pancreas: A Japanese Multi-Institutional Study

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Background: Recently, a consensus nomenclature and criteria were defined for classifying Intraductal papillary-mucinous neoplasms (IPMNs) of the pancreas into gastric, intestinal, pancreatobiliary, and oncocytic types based on morphology and immunohistochemical variations of mucin glycoproteins. The purpose of this study was to determine associations between the types and clinicopathological features in patients with IPMN.
Design: One hundred and eighty-five patients with IPMN collected from five Japanese institutions were retrospectively analyzed.
Results: Our series included 92 IPMNs of the gastric type, 71 of the intestinal type, 15 of the pancreatobiliary type, and seven of the oncocytic type. Statistically, the types of IPMN were significantly associated with the histological diagnoses, macroscopic types, invasion, and survival of the patients. The gastric-type IPMNs were likely to be diagnosed as benign (73/92, 79%), to be the branch duct type (64/92, 70%), to be non-invasive (82/92, 89%), and to have fair prognoses (96% in 5-year survival (5YS)). The intestinal-type IPMNs were likely to be diagnosed as malignant (61/71, 86%), to be the main duct type (42/71, 59%), to be non-invasive (45/71, 63%) but associated with colloid carcinoma when they were invasive (17/26, 65%), and to have less favorable prognoses (82% in 5YS). The pancreatobiliary-type IPMNs were likely to be diagnosed as malignant (15/15, 100%), to be the branch duct type (7/15, 47%), to be associated with invasive phenotypes (9/15, 60%), particularly with tubular adenocarcinoma (6/9, 67%), and to have poor prognoses (49% in 5YS). The oncocytic-type IPMN were likely to be diagnosed as malignant (7/7, 100%), to be the branch duct type (3/7, 43%), and to be non-invasive (5/7, 71%).
Conclusions: Evaluation of the types may help to predict the clinical course of patients with IPMN.

1388 Can Hepatocellular Carcinoma (HCC) Occur in Chronic Hepatitis B Virus (HBV) in the Absence of Cirrhosis?

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Background: While cirrhosis is a major risk factor for HCC, its pathogenesis has been also attributed to chronic injury, and viral carcinogenic effects. Reportedly, 10-50% of patients with HCC do not have cirrhosis. This would suggest that in the absence of cirrhosis, viruses may be directly oncogenic. The majority of cases of HCC in the world are due to HBV. Integration of HBV-DNA in the host genome can generate chromosomal instability and target the telomerase gene. HBV genes such as hepatitis Bx gene (HBx) have the portential to play a pivotal role in oncogenesis. The real functional consequences in hepatocarcinogenesis of these effects are, however, still unclear. The published data on HCC in the absence of cirrhosis do not take into consideration the now reported features of regression of fibrosis/cirrhosis. Regressed cirrhosis has certain histologic features that can be used to indicate the former presence of cirrhosis. The purpose of this study is to document the prevalence of overt and regressed cirrhosis, and lesser degrees of fibrosis, in livers with HCC and HBV, and to determine the proportion of HCC that occurs in absence of cirrhosis.
Design: 40 HCCs in resection specimens and explants in chronic HBV and background parenchyma were retrospectively reviewed. The tumor types were trabecular (usual-HCC). The patient demographics, number and size of tumors were recorded. Parenchymal fibrosis stage (F) was graded according to the METAVIR and Laennec systems. Hepatic parenchyma was evaluated by a panel of special stains. Fibrosis was evaluated using trichrome and reticulin stains.
Results: All patients but 1 had fibrosis. In 5 patients (8%), there was no evidence of overt or regressed cirrhosis (4 of these 5 had F1-F2). 9 patients without overt cirrhosis had features associated with regressed cirrhosis (approx 25%).
Conclusions: Usual-HCC in chronic HBV has cirrhosis or definite fibrosis in approx 97% of livers. The use of resected and explanted livers solves the issue of biopsy size and sampling effect in the staging of fibrosis. The pattern of fibrosis in livers without overt cirrhosis suggests that advanced fibrosis was previously present and had resorbed. Therefore many of the non-cirrhotic patients may have had overt cirrhosis in the past. Thus the risk of HCC appears to persist after regression of cirrhosis/fibrosis, but additional studies are required to shed more light on the magnitude of persistent risk.

1389 Renal Cell Carcinoma (RCC) Metastatic to the Pancreas: A Clinicopathologic and Immunohistochemical Study of 13 Cases

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Background: Pancreatic metastases of RCC are rare but represent a challenge to the surgical pathologist. The tumor needs to be distinguished from both primary pancreatic tumors and other morphologically-similar metastases with clear or granular cells. We summarize the experience of two tertiary care Canadian medical centers with emphasis on the updated immunoprofile of these lesions.

Design: Surgical pathology databases were searched in the period of July 1991 to June 2007 for all cases of RCC metastases to the pancreas. Surgical pathology reports, complete operative notes of current and previous surgeries, clinical information and follow up were reviewed. A representative metastatic tumor block was selected and a panel of antibodies was applied.

Results: Thirteen cases (M = 4; F = 9) were identified (clear cell = 11, sarcomatoid = 2). At the time of primary diagnosis, patients ranged from 44 to 82 years old with a median age of 71. The primary tumor originated from the right kidney in 5 cases and from the left in 8. All primary tumors were resected by radical nephrectomy. In 2 patients, the primary tumor and the pancreatic metastasis were synchronously diagnosed and resected. For the remaining patients, the interval between the primary and the pancreatic surgeries ranged from 24 to 298 months (median = 63) for 8 patients and was unknown in 3 cases. All pancreatic lesions were solitary and were resected by Whipple procedure in 5 cases, distal pancreatectomy with splenectomy in 3, and total pancreatectomy with or without splenectomy in 3. Only 2 cases were unresectable, and a frozen section and bypass surgery were performed. CD10 was positive in 12 cases, RCC marker (monoclonal antibody against normal human proximal tubular brush border) in 11, EMA in 9 and CK 20 in 2. Immunostaining for CEA, Heppar-1, CK 7 and inhibin was negative in all cases. One of the two cases negative for RCC marker was clear cell while the other was sarcomatoid; both cases were positive for CD10. On last contact, 3-136 months following pancreatic surgery (median = 31), 10 patients were alive with disease and 3 were dead of disease.

Conclusions: This series shows a female predominance, relatively long intervals between the primary and pancreatic recurrence in metachronous tumors and long term survival following pancreatic surgery. The suggested panel of antibodies reliably characterizes this tumor and helps distinguish it from other primary or metastatic tumors.

1390 Expression of High Mobility Group A (HMGA1) Proteins in Pancreatic Ductal Adenocarcinoma (PDA)

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Background: PDA is a lethal malignancy accounting for 〉150,000 deaths worldwide per year. Although some early molecular events contributing to PDA are well described, those facilitating tumor progression and metastasis are poorly understood. The HMGA1 oncogene encodes the HMGA1a and HMGA1b chromatin-binding proteins which function in regulating gene expression. HMGA1 was associated with metastases in an orthotopic mouse model of PDA, indicating that it may contribute to tumor progression. Our preliminary studies show that HMGA1a is overexpressed in cultured PDA cells compared to normal pancreatic tissue cells. Moreover, we found the highest levels in cells derived from metastases. We also demonstrated that forced overexpression of HMGA1 leads to a transformed phenotype in HPNE-K-RAS cells. Here, we investigate levels of HMGA1 protein in primary, human PDA from routine surgical specimens and correlate these levels with clinical outcome.

Design: Tissue microarrays (TMAs) were created using cores from routinely-processed, paraffin-embedded tissues sections. HMGA1 protein expression was determined by immunohistochemistry. TMAs included 125 PDA cases (4 cores/case) and representative normal tissues. Positive staining was identified as nuclear immunoreactivity and was scored using a 9 point scale based on the product of staining intensity (weak =1, moderate =2, strong =3) and staining extent (% of positive cells; 〈30% =1, 30-60% =2, 〉60% =3). The highest score per case was used for subsequent analyses.

Results: HMGA1 reactivity was identified in 123 of 125 PDA, with scores ranging from 1-9. Of positive cases, 22 (17.8%) displayed limited reactivity (score 〈3), 52 (42.3%) displayed moderate reactivity (score 3-6) and 49 (39.8%) displayed strong reactivity (score 〉6). HMGA1 reactivity was inversely associated with median survival, and patients with limited reactivity had longer survival than those with strong reactivity (〈3: 22.4 mos, 3-6: 18.2 mos, 〉6: 14.8 mos). Further, 49% of tumors with scores 〉6 were poorly differentiated, compared to 38% of those with scores 3-6 and 23% of those with scores 〈3.

Conclusions: We demonstrate that HMGA1 is expressed in PDA and correlates with shortened patient survival and poorly differentiated tumors. Our findings suggest that HMGA1 is an important molecular determinant of invasive disease and a potential therapeutic target in PDA.

1391 Molecular Alterations in Morphologic Subtypes of Pancreatic Intraductal Papillary Mucinous Neoplasm (IPMN) Are Distinct from Those Seen in Pancreatic Ductal Adenocarcinoma

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Background: IPMNs can be lined by several types of epithelium, including gastric (IPMN-G), intestinal (IPMN-I), oncocytic (IPMN-O) and pancreatobiliary (IPMN-PB). Based on an analysis of 157 resected IPMNs, we have recently reported differences in the clinicopathological characteristics of each morphologic subtype. However, molecular alterations associated with these subtypes have not been well characterized.

Design: Large core tissue microarray blocks were composed of 5 normal pancreata and 92 lesions consisting of 31 IPMN-G, 33 IPMN-I, 7 IPMN-O, 5 IPMN-PB, and 16 IPMN-associated invasive carcinomas (INVs) (7 colloid and 9 ductal carcinomas); 13 lesions showed mild dysplasia (MI), 28 moderate dysplasia (MD), 35 severe dysplasia (SD) and 16 INV. The expression of tumor suppressor genes (p16, p21, SMAD4), MMR proteins (MLH1, MSH2, MSH6, PMS2), MGMT and β-catenin were immunohistochemically evaluated and each core was scored according to previously described methods. The results were correlated with morphologic subtypes and neoplastic grades.

Results: There was no correlation between the expression of tumor suppressor genes and the morphologic subtypes. Loss of expression was seen only in lesions with SD and INV, especially of ductal phenotype, although at markedly lower rates compared to published data on conventional ductal adenocarcinomas (IDCs). Nuclear expression of β-catenin was present only focally in 3 lesions. Loss of expression of MGMT was seen in 2 cases and loss of MLH1 in 2 lesions of one case. Expression of MSH2, MSH6 and PMS2 was preserved in all lesions.

Conclusions: There are no differences in expression of common tumor suppressor genes, MMR proteins, MGMT or β-catenin between different morphologic subtypes of IPMN. Furthermore, the results support the hypothesis that IPMNs do not follow the established tumor progression pathway of IDC.

	p16*	p21#	SMAD4*
Morphologic subtype			
IPMN-G	3 (10%)	0	1 (3%)
IPMN-I	2 (6%)	1 (3%)	1 (3%)
IPMN-O	0	0	0
IPMN-PB	0	0	0
Colloid carcinoma	0	0	2 (29%)
Ductal carcinoma	3 (33%)	3 (33%)	4 (44%)
Grade			
MI	0	0	0
MD	0	0	0
SD	5 (14%)	1 (3%)	2 (6%)
INV	3 (19%)	3 (19%)	6 (38%)

* loss of expression, # overexpression.

1392 Expression of Inflammatory Mediators in Pancreatic Intraductal Papillary Mucinous Neoplasm (IPMN)

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Background: IPMNs can be lined by several types of epithelium, including gastric (IPMN-G), intestinal (IPMN-I), oncocytic (IPMN-O) and pancreatobiliary (IPMN-PB). We have reported that IPMN-I is more commonly observed in the proximal main pancreatic duct compared to other subtypes. Based on the results, we hypothesized that the inflammation of the ductal epithelium secondary to retrograde exposure of bile and/or acid may contribute to development of the intestinal morphology and neoplastic progression.

Design: Large core tissue microarray blocks were composed of 5 normal pancreata and 92 lesions consisting of 31 IPMN-G, 33 IPMN-I, 7 IPMN-O, 5 IPMN-PB, and 16 IPMN-related invasive carcinomas (INVs: 9 associated with IPMN-I [INV-I] and 7 others [INV-NI]); 13 lesions showed mild dysplasia (MI), 28 moderate dysplasia (MD), 35 severe dysplasia (SD) and 16 INV. Sections were immunohistochemically stained with NFkappaB (p65) and COX-2 and each core was scored as 0-3 and 0-1, respectively. The results were correlated with the morphologic subtypes and dysplastic grades.

Results: The average score of p65 stain was 2.00, 2.34, 1.93 and 2.45 in IPMN-G, IPMN-I, IPMN-O and IPMN-PB, respectively. It was 2.13, 2.13, 2.27 and 1.96 in MI, MD, SD and INV, respectively. p-65 expression was significantly increased in IPMN-I than in IPMN-G ($p < 0.005$) and IPMN-O ($p < 0.05$). It was also increased in IPMN-PB than in IPMN-O ($p < 0.05$). There was no correlation between p65 expression and neoplastic grades. Interestingly, INV-I showed stronger p65 expression than INV-NI (2.10 vs. 1.73, p= 0.052). COX-2 overexpression was seen in 45%, 39%, 86% and 80% of the IPMN-G, IPMN-I, IPMN-O and IPMN-PB groups, respectively. It was noted in 46%, 43%, 54% and 75% of the MI, MD, SD and IVC groups, respectively. COX-2 overexpression was less frequent in IPMN-I than IPMN-O ($p < 0.05$). When only SD cases were compared, IPMN-I infrequently overexpressed COX-2 compared to IPMN-O ($p < 0.05$) and IPMN-PB ($p < 0.1$). COX-2 overexpression was more frequent in IVD than in MD ($p < 0.05$); however, there was no difference in COX-2 expression between MI, MD and SD.

Conclusions: Both IPMN-I and IPMN-PB appear to be associated with activation of NFkappaB, a key inflammatory mediator. However, in IPMN-I, the activation was not linked to COX-2 overexpression that has been documented in conventional ductal adenocarcinoma. The infrequent COX-2 overexpression may be associated with the low progression rate of IPMN-I, and ultimately a less aggressive behavior.

1393 Mismatch Repair Proteins in Pancreatic Endocrine Neoplasms

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Background: Among gastrointestinal endocrine neoplasms, methylation of mismatch repair (MMR) genes is largely restricted to pancreatic endocrine neoplasms (PEN). The aberrant promoter methylation of the MMR gene, MLH1, in PENs has been associated with improved survival. MGMT-mediated repair of DNA is unique compared with other DNA repair pathways. The absence of MGMT expression appears to define patients with PENs who achieve significant benefit from the drug temazolamide. This study investigates the status of MMR proteins and MGMT in a large cohort of PENs, and characterizes their clinical and histopathological correlates.

Design: PETs were obtained from 67 patients who underwent surgical resection. The demographic, clinical, pathologic, and eventual outcome of these cases was

recorded. All tumors were classified according to the current WHO classification system. Immunohistochemical expression of MLH1, MSH2, MSH6 and MGMT was evaluated on a tissue microarray. A CD3 stain was performed and quantitative analysis of intratumoral T-cells was recorded (Aperio Scanscope CS system, Aperio Technologies, CA).

Results: The cohort was composed of 34 males and 33 females with a mean age 55.42 years and a median age of 55 years. Loss of MLH1 expression was documented in 8/63 (13%) cases and MGMT expression in 14/66 (21%) cases. MLH1 and/or MGMT expression was lost in 19/63 (30%) cases. Loss of MGMT expression correlated with an elevated Ki67 labeling index (p=0.003). The number of intratumoral lymphocytes did not correlate with MMR or MGMT expression. Dual MGMT and MLH1 negative tumors were more likely to be categorized as well differentiated endocrine carcinomas (31%) than tumors demonstrating intact expression of either protein (13%) (p=0.09, NS). MSH2 and MSH6 protein expression was intact in all tumors studied.

Conclusions: In accordance with prior literature a high proportion of PENs demonstrate loss of MLH1 and/or MGMT protein expression. Unlike the colonic carcinoma paradigm, loss of MMR proteins does not appear to correlate with the presence of intratumoral lymphocytes and may not confer a survival advantage in PENs.

1394 The Potential Role of Myofibroblast Presence and Transforming Growth Factor-β1 in Two Different Models of Experimental Cirrhosis

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Background: Progress of chronic liver failure is related to liver fibrosis, which originates mainly from Ito cells that display myofibroblastic features. This study a) evaluates the role of myofibroblasts and assesses the source of the profibrogenic cytokine TGFβ1 production in cirrhosis in rats, and b) investigates whether the model used for cirrhosis induction affected the presence of the aforementioned factors.

Design: The study comprised 102 male Wistar rats divided in 3 groups: A (n=48): CCl4 injection (intraperitoneally 2ml/kg/BW-1:1 vol in corn oil twice weekly), B (n=48): thioacetamide administration in drinking water (300 mg/L) for 3 months and C (n=6) controls. Rats of group A were sacrificed at 4,8,12 weeks (w) and rats of group B in 1,2,3 months (mo). SGPT values were measured in blood samples. Liver tissues were subjected to: **a)** real time PCR/Western blot (TGFβ1 mRNA/protein), **b)** immunohistochemistry [αSMA expression (myofibroblasts presence) and TGFβ1 protein distribution] and **c)** CISH (TGFβ1 and procollagen-I mRNA distribution). Results were expressed following image analysis.

Results: Liver sections from groups A and B showed bridging fibrosis in 8w and 2mo, and cirrhosis in 12w and 3mo, respectively. The results from groups A and B, regarding expression of the factors studied, were comparable. In both groups: **a**) PCR and Western blot showed that TGFβ1 mRNA/protein expression were higher towards advanced fibrotic stages ($p<0.01$ for each group), **b**) αSMA+ cells were detected in lobules and fibrous bands. At 12w and 3mo the increase for αSMA+ cells, compared to controls, was 63.2 and 67.4 folds, respectively, for lobules ($p<0.05$). Procollagen-I mRNA was co-localized to αSMA+cells in fibrous bands, showing that activated myofibroblasts lead to increased collagen deposition in cirrhosis in rats. TGFβ1 mRNA/protein were detected in fibrous septa and within hepatocytes close to fibrous septa. A direct correlation was seen between %αSMA+ and %TGFβ1+ cells with fibrosis degree and SGPT values ($p<0.05$ in each case for each group).

Conclusions: This study shows that in experimental cirrhosis (irrespective of the model used for cirrhosis induction), **a)** activated myofibroblasts are responsible for increased collagen production and **b)** activated myofibroblasts and hepatocytes are involved in the production of TGFβ1. Both processes may hold a definite role during progress of liver failure and they are implicated in the pathogenesis and progress of liver fibrosis.

1395 BAX/BCL-2 Ratio and Caspase-3 Expression in Two Different Models of Experimental Cirrhosis

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Background: Apoptosis has been linked to liver cell depletion and ensuing liver fibrosis. Bax/bcl-2 ratio has been considered as the best regulator of apoptosis. In this study we a) assessed the alterations of bax/bcl-2 ratio in relation to changes in the apoptosis co-ordination enzyme caspase-3, in 2 different models of experimental cirrhosis and b) investigated whether the model used for cirrhosis induction affected the alterations of the aforementioned factors.

Design: The study comprised 102 male Wistar rats divided in 3 groups: A (n=48):CCl4 injection (intraperitoneally 2ml/kg/BW-1:1 vol in corn oil twice weekly), B (n=48): thioacetamide (TAA) administration in drinking water (300 mg TAA/L) for 3 months and C (n=6) controls. Rats of group A were sacrificed at 4, 8 and 12 weeks (w) and rats of group B in 1, 2 and 3 months (mo). SGPT values were measured in blood samples. Liver tissues were evaluated for **a**) fibrosis degree, **b)** Bax, Bcl-2 mRNA (real time RT-PCR), **c)** Bax, Bcl-2 protein (Western blot), and distribution (immunohistochemistry), **d)** caspase-3 activity (substrate cleavage assay), and **e)** apoptosis (TUNEL method). Results were expressed following image analysis and the bax/bcl-2 ratio was calculated.

Results: Rats of group A and group B developed ascites in 12 w and 3 mo respectively. Liver sections from groups A and B showed bridging fibrosis in 8 w and 2 mo, and cirrhosis in 12 w and 3 mo, respoectively. The results regarding the expression of the factors studied, were comparable: Bax mRNA was significantly increased and Bcl-2 mRNA decreased (compared to controls) towards 12 w (+252% and -61%) and 3 mo (+267% and -54%). Similar results were recorded for bax and Bcl-2 proteins (+371% and -25% by 12w and +387% and -32% by 3rd mo). The ratios of Bax/Bcl-2 mRNA and protein were increased at all time points (towards 12w and 3 mo) in both groups. These ratios for mRNA and protein correlated, in both groups, with *up-regulated caspase-3 activity* ($p<0.01$ for each group), *apoptosis* ($p<0.01$ for group A and $p<0.05$ for group B), *SGPT values* ($p<0.01$ for each group), and degree of fibrosis ($p<0.01$ for each group).

Conclusions: This study shows that in experimental cirrhosis, (irrespective of the model used for cirrhosis induction), bax/bcl-2 ratio contributes to caspase-3 activation and increase of liver cell apoptosis. These results may have prognostic and therapeutic implications in chronic liver failure.

1396 Ductular Hepatocytes Constitute a Cell Population Different from Oval Cells, Express HNF4α and Contribute to Liver Regeneration in Experimental Acute Liver Failure

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Background: Liver regeneration following liver damage is mainly mediated by proliferation of intact mature hepatocytes; when the latter is suppressed regeneration is mediated by oval cell (OC) proliferation. A previous study **a)** showed that in human livers with submassive necrosis, ductular hepatocytes [DH-bipotential progenitor cells expressing both hepatocytic (HEPAR) and biliary (Cytokeratin 19-CK19) markers], express HNF4α (a factor that regulates the expression of genes of hepatocyte differentiation) and contribute to liver regeneration and **b)** stated that human DH are similar to rodent OC. In this study and, in a model of experimental acute liver failure, we attempted to find out whether DH **a)** are present during liver regeneration, **b**) are similar or different to OC, and **c)** express HNF4α.

Design: Wistar rats (n=120) received simultaneously LD50 doses of allyl-alcohol (intraperitoneally 0.05ml/kg) and CCL4(rhinogastric 1.9ml/kg) and sacrificed 2, 4, 6, 12, 24, 48, 81, and 153 hr, after. Liver tissues were evaluated for the presence of DH and OC and the immunohistochemical expression of HNF4α. *Morphometric analysis: DH:* cells located mainly at the periportal areas, lined tubular-shaped structures, and were CK19+ *and* HEPAR+. *OC:* cells with ovoid nuclei and scant cytoplasm which were CK19+ *and* HEPAR-. Cell counts were performed manually at a X400 magnification, on five non-overlapping different fields per section, using a 10X10-microscope grid.

Results: The animal death rate was 80% at 48 hr. Liver sections showed areas of combined (periportal+pericentral) parenchymal necrosis, peaking at 48 hr. DH were present in all liver sections; they were located in periportal areas and near the nodules of regenerative hepatocytes. The percentage of HNF4α+DH ranged from 6.4% to 47.2% (mean 18.3%). HNF4α+DH numbers were higher in periportal areas when compared to midzonal and pericentral areas (25.6% vs. 8.5% vs 6.2%-$p<0.001$). Regenerative hepatocytes near necrotic areas showed lower HNF-4α expression, implying that intact hepatocytes may induce hepatocyte differentiation in adjacent DH. Triple stain showed that DH co-expressed CK19/HEPAR/HNF4α. OC were present mainly in periportal areas and did not express HNF4α.

Conclusions: In rat livers with combined periportal and pericentral necrosis, DH are present and constitute a different cell population than OC. The expression of HNF4α in DH supports the concept that DH differentiate into hepatocytes and contribute to liver regeneration.

1397 DNA DNA Hypermethylation of 43 CpG Island Loci and DNA Hypomethylation of Repetitive Elements in HCC and Their Inter-Relationship and Prognostic Implication

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Background: Aberrant DNA methylation changes, characterized by focal hypermethylation and generalized genomic hypomethylation, are common findings in human cancers, regardless of tissue type. Hepatocellular carcinoma (HCC) has been known to have both aberrant methylation changes but the relationship between hypermethylation and hypomethylation remains unclear in HCC. This study aimed to determine the relationship between DNA hypermethylation and hypomethylation and their prognostic implications in HCC and the association of DNA methylation changes with clinicopathologic factors.

Design: One hundred seventy three hepatocellular carcinomas were analyzed for their methylation status for 43 CpG island loci and three repetitive DNA elements (*LINE-1*, *ALU*, and *SAT2*) using quantitative methylation-specific PCR or COBRA assay.

Results: Most frequently methylated loci (>40% of HCC cases) included *RASSF1A*, *APC*, *CYP27B1*, *DLEC1*, *MINT31*, *SOCS1*, *HOXA1*, *GSTP1*, *PTGS2*, *CDKN1C*, *SOCS3*, *MINT2*, *p16*, *RUNX3*, *IGF2*, *TWIST1*, and *CACNA1G*. HCC cases with methylation of *APC* or *p16* showed significantly lower methylation levels of repetitive DNA elements than those in HCC cases without methylation of the respective locus, whereas HCC cases with methylation of *DLEC1*, *TWIST1*, *CRABP1*, *GRIN2B*, *CCND2*, *CDH13*, *MT1G*, *PTGS2*, *SCGB3A1*, *SFRP1*, or *SYK* showed significantly higher methylation levels of repetitive DNA elements than those in HCC cases without methylation of the respective locus. The number of methylated genes in HCC was closely associated with background liver and the more cirrhotic, the more number of genes methylated. Of 43 CpG island loci, three loci (*APC*, *CYP27B1*, and *SYK*) showed significant association with patients' survival; methylation of *APC* or *CYP27B1* was associated with better survival, whereas that of *SYK* was associated with poorer survival.

Conclusions: The fact that varying relationships between hypermethylation of individual CpG island locus and hypomethylation of repetitive elements suggests that they are independent events and not mechanically linked. The findings suggest that the methylation status of HCC may be influenced by background liver. Some CpG island loci may serve as useful tumor marker for prognostication of HCC patients.

1398 Metastatic Pancreatic Cancer to the Lung: A Potential Mimicker of Primary Bronchioloalveolar Carcinoma of the Lung

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Background: Patients with a history of pancreatic ductal adenocarcinoma may present with a new lung mass. The distinction between a primary lung adenocarcinoma and metastasis from a pancreatic primary can be diagnostically challenging. This distinction has important therapeutic and clinical implications.

Design: Eighteen patients with lung nodule(s) and a history of pancreatic ductal adenocarcinoma were collected as part of a rapid autopsy program for pancreatic cancer. These patients had complete clinical follow-up of multiple clinical parameters. Additionally, a total of 5 patients with a new lung mass and a history of pancreatic ductal adenocarcinoma were identified in our surgical pathology files (1997-2007). The morphological characteristics of the lung nodules were compared to the primary tumor and other metastatic sites, if present.

Results: In 23 patients with a known primary pancreatic ductal adenocarcinoma, we examined the morphological features of metastatic lesions in the lung. In 5 of 23 cases (22%), the metastatic adenocarcinoma exclusively grew along the alveoli (lepidic growth), with a mucinous-type epithelium, and showed no evidence of a desmoplastic response. In these cases, the cytologic and cellular features were different than the original primary tumor, as well as other metastatic sites. Most significantly the morphology in the cases demonstrating lepidic growth was similar to the morphology observed in primary bronchioloalveolar carcinomas. In the remainder of cases, the morphology of the metastasis was similar to the original primary tumor.

Conclusions: Metastatic pancreatic carcinoma in the lung can act as a mimicker of primary bronchioloalveolar carcinoma of the lung. Due to the clinical and prognostic implications, distinguishing the bronchioloalveolar pattern of metastasis, in patients with a history of pancreatic ductal adenocarcinoma, from a new lung primary is of diagnostic importance. Further studies utilizing immunohistochemistry are necessary to help make this distinction.

1399 Autoimmune Hepatitis in Patients with Primary Sjögren's Syndrome: A Series of Two-Hundred and One Patients

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Background: Primary Sjögren's syndrome (pSS) is a systemic autoimmune disorder characterized by the simultaneous presence of keroconjunctivitis sicca and xerostomia in the absence of other connective tissue diseases. Although pSS is an autoimmune exocrinopathy, the involvement of non-exocrine organs is frequently reported. Two small case series have previously reported that the prevalence of autoimmune hepatitis (AIH) in patients with pSS ranges widely from 4% (2/45) to 47% (8/17). The prevalence of AIH in the general population is approximately 0.0004%.

Design: Two-hundred and one patients were identified from a pSS database. Anatomic pathology computer records were queried for each of these patients and all diagnoses were identified and reviewed. In select patients, relevant clinical history and laboratory values were also identified, including history of drug or alcohol use, liver function tests, total serum globulin, autoantibody seropositivity, and hepatitis viral markers.

Results: Of the two-hundred and one patients, two (1%) were identified as having definite AIH, as defined by the International Autoimmune Hepatitis Group diagnostic criteria. The two patients, both women, were 28 and 53 years-old at the time of diagnosis. The elder patient required orthotopic liver transplantation six years after diagnosis. Neither patient demonstrated evidence of primary sclerosing cholangitis or primary biliary cirrhosis. Patients with serologic evidence of viral hepatitis were excluded. Secondary findings included a patient with eosinophilic gastritis and another patient diagnosed with non-alcoholic fatty liver disease.

Conclusions: We found that the prevalence of AIH in patients with pSS is 2500 times that of the general population. Two previous small case series have documented that the prevalence of AIH in patients with pSS ranges widely from 4% (2/45) to 47% (8/17). Our larger series has demonstrated the prevalence to be approximately 1%. This likely represents a more accurate estimate of the true prevalence of AIH in patients with pSS.

1400 Quantitative Analysis of CpG Island Hypermethylation in Extrahepatic Cholangiocarcinoma, Biliary Dysplasia and Normal Bile Ducts

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Background: Biliary dysplasia is the premalignant lesion of bile duct which develops to extrahepatic cholangiocarcinoma (ECC). In the multistep carcinogenesis sequence, morphologic progression of the lesion is associated with accumulation of genetic and epigenetic changes. But, in ECCs, there is no published data about epigenetic changes through disease progression. The objective of this study is to generate methylation profiles of multiple CpG island loci in biliary dysplasia and to compare it with that of ECCs and normal bile ducts.

Design: A total of 31 ECCs, 28 bilary dysplasias and 31 normal bile duct samples from surgical specimens of ECC patients were investigated for this study using real time PCR-based MethyLight technology. We analyzed six CpG island loci (*HPP1, HOXA1, NEUROG1, RUNX3, RASSF1A and APC*) which were differently methylated in ECCs and normal bile ducts in the previous study using methylation-specific PCR.

Results: In biliary dysplasias, a high methylation frequency was observed in *HPP1* (82%), *HOXA1* (54%) and *NEUROG1* (29%). But it was lower than that of ECCs (90%, 50% and 61%, respectively) and higher than that of normal bile ducts (57%, 54% and 0%, respectively). Differences of methylation frequencies were statistically significant at all six CpG island loci (p=0.001~0.005) in ECC, dysplasia and normal bile ducts. There was also a statistically significant (p<0.001) trend for stepwise increase in positive methylation rate (PMR) values which represents methylation levels.

Conclusions: This is the first study about methylation profiles of biliary dysplasia. Six CpG island loci, which were highly methylated in ECCs, were found to be significantly methylated in biliary dyplasia compared to those of normal bile ducts. This result supports, as well as histopathologic and genetic changes, epigenetic changes in extrahepatic bile duct neoplasm progress through multistep process.

1401 Fibronectin Receptor: A Unique Malignant Biomarker of Pancreatic Carcinoma

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Background: Carcinoma of pancreatic ductal origin is notable for eliciting a strong desmoplastic stromal reaction. The responsible signals for this pathologic behavior may affect cancer progression and aggressive behavior. Upregulation of fibronectin receptor (integrin beta-1, FNRB) a member of the human adhesion protein receptor family, and stromal cell derived factor 4 (SDF4), a calcium binding signal transduction protein, have been identified in pancreatic carcinoma cell lines using proteomics and global gene expression arrays. The aim of the present study is to systemically analyze expression of these genes and their relationship to pathologic behavior and prognosis in pancreatic cancer.

Design: 82 cases of pancreatic ductal adenocarcinoma spanning 15 years were reviewed (archived at Northwestern Memorial Hospital). All were analyzed for both anti-SDF-4 and anti-FNRB reactivity by an immunohistochemical approach with proper positive and negative controls. The biologic activity was correlated to clinical data.

Results: 91% of pancreatic carcinomas (73 of 80 cases) showed mild to strong staining for FNRB. Positive staining was confined to the carcinoma cell membrane. Semi-quantification ranged from mild (1+) to strong (3+) with discontinuous membrane staining in 1+ and 2+ to complete circumscription in 3+. Extent of FNRB staining was markedly correlated with patient survival:16 cases with 3+ staining averaged 273 days survival; 30 cases with 2+, 689 days; 27 cases with 1+, 903 days. Similar correlation was also identified for tumor stage and grade. Furthermore, in patients who had survived 1000 days or greater (>2.5 y), 100% (16 of 16 cases) had scored less than 3+ for FNRB. In addition, in 8 of 16 cases with 3+ staining of adjacent desmoplastic stroma but 0-2+ staining of tumor cells, stromal component contributed to decreased survival (less than 1 yr). SDF4 was extensively positive in the cytoplasm of tumor, duct epithelium, inflammatory cells, and fibroblasts, and did not show correlation with pathologic behavior of carcinoma.

Conclusions: Our study demonstrates that increased fibronectin receptor positivity is associated with high tumor stage and low survival and decreased fibronectin receptor expression correlates with longer survival. It also implicates a relationship between tumor cell and adjacent stroma positivity. These results indicate fibronectin receptor may serve as a potential malignant biomarker for pancreatic cancer and necessitates further investigation of its role in cancer behavior.

1402 Lessons Learned from 5 Year Survivors of Pancreatic Adenocarcinoma: A Critical Look at the AJCC Staging System

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Background: The overall 5-year survival for pancreatic adenocarcinoma (PDAC) following surgical resection is 12-19%. The American Joint Committee on Cancer (AJCC) Cancer Staging Manual 6th Edition is used to stratify stage-specific survival. Recent studies have reported conflicting results regarding the predictive value of the T classification. Our aim was to evaluate the prognostic impact of the T classification utilizing our PDAC 5-year survivors.

Design: Surgical pathology files and clinical records were reviewed to identify PDAC patients who underwent curative surgical resection and survived for 5 or more years. Gross and microscopic appearances of the tumor were reviewed for each case. Grade, TNM stage, clinical stage, nodal status, margin status, and tumor site and size were recorded. Involvement of the peripancreatic soft tissues, bile duct and duodenum, features indicative of pT3 lesions was also noted. Staging was based upon the AJCC Cancer Staging Manual 6th Edition.

Results: Fifty-six 5-year survivors were identified. Complete clinical and pathology data were available for 28 patients. Seven patients with periampullary adenocarcinoma or cholangiocarcinoma were excluded. Our final cohort consisted of 9 males and 12 females (median age 67 years, range 33-80). These patients underwent either a pancreaticoduodenectomy (n=19) or a distal pancreatectomy (n=2). The median size of tumor was 2.5cm (range 0.2-5). The PDACs were well differentiated (n=10), moderately differentiated (n=9), and poorly differentiated (n=2). The bile duct was involved in 5, and the duodenum in 8 of 19 Whipple specimens. The peripancreatic adipose tissue was involved in 14 of 21 cases. The majority of cases were T3 lesions (n=15, 71.4%). T1 and T2 lesions were equally represented (n=3, 14.3%). There were 3 patients with stage IA and IB disease (14.3%), 10 with IIA disease (47.6%), and 5 with IIB disease (23.8%). Five specimens (23.8%) had at least one positive margin. The majority of patients had node negative disease (n=13, 61.9%)(median number of nodes=9). Seven patients (33.3%) had positive nodal status, all with T3 lesions. The median survival was 75.6 months (6.3 years).

Conclusions: The current T classification of the AJCC staging system does not accurately identify PDAC 5-year survivors following curative surgical resection. Additional studies are required to refine the staging system to more accurately reflect the clinical behavior of these tumors.

1403 Identification of Serum Markers in Cirrhosis – A Proteomics Based Approach

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Background: Steatohepatitis, fibrosis, and cirrhosis are common outcomes in alcoholic hepatitis as well as NASH. Liver biopsy is considered the gold standard for assessing progressive fibrosis. An invasive procedure such as liver biopsy has potential complications to the patients as well as has impact on resources. Hence it is important to identify potential novel markers, which may be useful to predict progression of liver disease in alcoholic and non-alcoholic hepatitis as well as in other causes of cirrhosis.

Design: Serum from patients with biopsy proven cirrhosis of various etiologies; eg, NASH (n=9), alcohol (n=5), and others (n=6), who underwent liver transplant over a period ranging from 2004-2006, were utilized for retrospective analysis. Serum samples were also collected from a group of volunteers without any known liver disease (n=13). All serum samples were subjected to proteomics analysis using Luminex technology, measuring 25 cytokines that are known inflammatory, angiogenic, and fibrosis markers. The specific protein levels were statistically analyzed.

Results: Of the 25 markers examined, 13 were shown to be significantly elevated (2-30 fold) when the levels in the cirrhotic patients were compared with the control group (Table 1). These cytokines include adipokines, markers of apoptosis, inflammation and angiogenesis. Serum of NASH patients showed elevated HGF, β FGF, IL1 β levels compared to alcoholic cirrhosis. Compared to 'others' both NASH and alcohol group showed elevated MMP2, sFAS ligand and reduced PDGFbb levels. There were no correlations between associated clinical histories (i.e. obesity, diabetes, liver function tests) and the level of serum markers.

Serum markers and their level-fold increase

Apoptosis markers	
SFAS	3x
TNFR-1	6x
TNFR-2	4x
Angiogenic and growth factors	
TGF-beta	5x
HGF	4x
PDGF-bb	2x
Adipokines	
Adiponectin	2x
Resistin	3x
Proteinases	
MMP-1	3x
MMP-2	2x
MMP-9	8x
Cytokines	
IL-1ra	30x
IL-6	10x

Conclusions: The data from this study indicate that a large number of serum markers involved in various processes such as apoptosis, fibrosis and angiogenesis are altered in cirrhosis and a panel of such markers may potentially be useful in assessing degree of fibrosis in patients with chronic liver diseases. However, further prospective studies with larger sample size will be useful to identify specific disease-associated cytokines.

1404 Presence and Diagnostic Significance of CD21+ Follicular Dendritic Cells in Liver Biopsies with Hepatitis C and Acute Allograft Rejection

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Background: Portal chronic inflammation with lymphoid aggregates/follicles is a well recognized histologic feature of chronic hepatitis C (HCV). These follicles consist of a central zone of B lymphocytes, a follicular dendritic cell (FDC) network and a surrounding zone of T lymphocytes. In the post-transplant (PT) setting, the presence of acute rejection (AR) may be accompanied by findings suggestive of concurrent HCV. Assessment of both processes is important due to the impact on clinical management. In this study our aims were: (1) to define the presence and distribution of CD21+ FDCs in AR and compare the findings with those in HCV, and (2) to determine whether the findings would be useful in the differential diagnosis of recurrent HCV versus AR.

Design: Paraffin immunoperoxidase studies were performed on 50 core biopsies: 20 cases with AR (6 mild, 14 moderate; 7d –84mo PT), 10 with recurrent HCV (2–77 mo PT), 10 with mixed features of HCV/AR (3 to 52 mo PT) and 10 with non-transplant HCV. For the 20 cases with only AR, the original liver diseases included cryptogenic cirrhosis (7), HCV (3), ETOH (3), HCV/ETOH (1), PSC (3), PBC (1), AIH (1) and acetaminophen toxicity (1). The diagnosis was confirmed in all biopsies using established criteria. Four micron sections were stained with an antibody to CD21 (Clone 2G9) using the Ultra Vision Labelled Polymer Detection System (Lab Vision, Fremont CA). Immunostains were reviewed by 2 pathologists (MK, AK) for the presence of CD21+ FDCs, identified by intense membranous staining.

Results: CD21-positive dendritic cells were present in 5 of 10 cases of non-transplant HCV, 2 of 10 cases of post-transplant HCV and 1 of 10 cases of HCV/AR. None of the 20 cases of AR showed immunoreactive FDCs. In the positive cases, the FDC network was only localized to the central zones of dense portal lymphoid aggregates.

Conclusions: 1) The presence of FDC+ lymphoid aggregates in post-transplant biopsies of HCV patients suggests recurrent disease, with or without AR 2) FDC+ lymphoid aggregates are less common in post-transplant recurrent HCV compared to non-transplant chronic HCV.

1405 Hepatocellular Adenomas in a US Center: Clinical-Pathologic Correlations Utilizing a Recently Proposed Classification

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Background: A proposed classification of hepatic adenomas (HA) based on molecular and clinico-pathologic correlations (Hepatology 2006;43:515) includes tumoral steatosis (in HNF1αmutated HA), inflammatory changes (in telangiectatic HA), and risk of hepatocellular carcinoma (increased in Bcatenin (BC)- activated HA). Correlations have now been shown with immunohistochemistry (IHC) from a French center (Hepatology 2007, in press).

Design: 13 cases of resected HA were reviewed; IHC was performed and evaluated per the French protocol.

Results: IHC results led to 4 grps: HNF1αmutated (LFABP neg), n=4; BC activated (BC and GS pos), n=1; Inflammatory (SAA pos),n=5; no specif immunophenotype, n=3. No diffs were seen in clinical or path findings except as noted: Clinical: 100% of the 13 adenomas were in women; 12 were Caucasian, 1 was African-Am. 12 were <50 yr; 10 were overweight or obese. OCA use was + in 80%. The majority presented with pain and had >5cm tumors. 4 cases had adenomatosis, 2 were HNF1αmutated. (p=0.05) Pathology: Tumor steatosis (>1/3) was found in 5; all the HNF1αmutated and 1 inflammatory HA. (p=0.02). Hemorrhage was seen in 6. Atypia was present in 5: 2/4 HNF1αmutated, 1/1Bcatenin-activated, 2/5 inflammatory HA. IHC reactivity was not homogenously distributed in HA.

Conclusions: The clinical findings in this series are representative both of HA (women with OCA use, presentation with pain) and of our patient population (overweight). The ethnic distribution of Cauc > AA is unexplained. While the majority (61%) of HA were single, adenomatosis cases were either HNF1αmutated or negative for IHC; none were SAA+ or BC activated. 50% of HNF1αmutated HA were multiple. All mutated HA had tumor steatosis; only 1 HA in another category did (an SAA pos tumor). The significance of atypia is not known. While predictable, because of inhomogeneity of IHC, careful interpretation, particularly in biopsy material, is recommended.

Patterns of IHC

Antibody	Normal Liver	Tumor Reactivity
LFABP	Diffusely +, cytoplasmic	HNF1α Mutated: Absence of Staining
SAA	+/-, granular, pericanalicular	Strongly +; clusters of cells; granular, pericanalicular
Glutamine Synthetase	Perivenular hepatocytes; cytoplasmic	Not restricted to "zone"
βCatenin	Diffusely + hepatocyte membranes; bile duct cytoplasm	Tumor nuclei +/- cytoplasmic

1406 Observer Variability in the Histopathologic Diagnosis of Hepatocellular Nodules

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Background: Improved surveillance of cirrhotic patients has resulted in increased detection of small high-risk nodules (HN). The gold standard now is the histologic diagnosis (dx) to distinguish among regenerative nodule (RN), dysplastic nodule (DN) and hepatocellular carcinoma (HCC), which is often difficult. However, the variability in interpretation of these nodules among pathologists is unknown. We undertook an observer variability study among experienced hepatopathologists.

Design: Two of the authors (HLW, JKL) selected 36 HNs from liver explants or resections from one institution. The nodules had a mean diameter of 10 mm (range: 3-23 mm). H&E and matched reticulin stain from each nodule were studied by 7 experienced hepatopathologists for their dx. A second review, at least 2 weeks later, was performed and dx criteria were provided with their dx. Inter- and intrarater variabilities were analyzed using unweighted κ and intraclass correlation coefficient statistics. Concurrence was defined as identical dx in 11 of 14 readings (2 dx by 7 pathologists).

Results: Concordant dx was achieved in two-thirds of the HNs. The inter-rater variability was "almost perfect" (κ=0.927) and the intra-rater variability was at least "substantial" (κ range: 0.694-0.972). The agreement for the dx of HCC was "almost perfect" (κ=0.802, CI: 0.77-0.83). In contrast, concordant dx of DN was achieved only on 1 nodule, while the mean number of DN dxs per review was 7.3±4.8 (range: 2-19 of 36 HNs). The dxs of 12 discrepant cases varied from RN, DN to HCC. Among them, 4 were classified as HCC, 2 DN and 4 RN by the majority of dxs (>7 of 14 dxs). The other 2 were classified as DN or RN each by 7 dxs.

Conclusions: Histopathologic distinction between HCC vs. non-HCC, the most critical distinction, was highly agreed upon among experienced hepatopathologists. However, the substantial variability in diagnosis of DN highlights the challenges in this dx, which are likely compounded when examining needle biopsy specimen. Our findings emphasize the need for improved definitions and diagnostic criteria and for development of reliable adjunct diagnostic tests.

1407 Immunoprofiling of Hepatocellular Nodules

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Background: Histologic diagnosis (dx) of high-risk nodules (HNs) in cirrhotic livers can be a challenge. Suggested immunomarkers may show poor reproducibility, partly owning to the accuracy of histologic dx upon which the markers are evaluated. We undertook a concurrence approach for histologic dx in order to evaluate the utility of a panel of immunomarkers.

Design: After two evaluations of 36 HNs by 7 experienced hepatopathologists, 24 diagnoses were concurrent for 11 regenerative nodules (RNs), 1 dysplastic nodule (DN) and 12 hepatocellular carcinomas (HCC). Immunostains for glypican 3 (GPC3), hsp70, clusterin, glutamine synthetase (GS), p53, Ki-67, CD34, GFAP, AMACR and β-catenin were evaluated by two authors (HLW, JKL) and staining patterns of HNs and surrounding cirrhotic liver recorded. The sensitivity (SEN) and specificity (SP) of the individual markers as well as multi-stain immunoprofile validity was calculated.

Results: Only 3 markers showed noticeable difference between HNs vs. surrounding livers. GPC3+ was defined as strong cytoplasmic staining vs. focal/weak expression seen in 25% of the cirrhotic livers. Clusterin+ was characterized by a strong exaggerated canalicular pattern vs. normal delicate canalicular staining. CD34+ was defined as strong and diffuse sinusoidal staining vs. periseptal staining noted in cirrhotic nodules. The SEN, SP, positive, and negative predictive values in % were 75, 75, 75, 75 for GPC3; 92, 100, 100, 92 for clusterin; and 33, 83, 66, 55 for CD34, respectively. Only 3 HCCs showed an increased Ki-67 labeling index. AMACR, β-catenin, GS and Hsp70 showed no difference between HNs and surrounding livers. GFAP and p53 were negative in all cases.

Conclusions: Exaggerated canalicular pattern of clusterin staining and strong cytoplasmic GPC3 expression are sensitive and specific markers to distinguish HCC from non-HCC nodules. The staining profile of DN appears to resemble that for RN but study of a larger number is required to solidify this observation.

	Concordant Diagnosis			Discordant Diagnosis			
n=	12 HCC	11 RN	1 DN	Favor HCC 4	Favor RN 4	Favor DN 2	RN vs. DN 2
GPC3	9 (75%)	3 (27%)	0	1	1	0	0
Clusterin	11 (92%)	0	0	0	0	0	0
CD34	4 (33%)	1 (9%)	1	2	0	0	0

1408 The Akt Pathway in Angiogenesis of Hepatocellular Carcinoma: A Immunohistochemical Stain Based Analysis

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Background: Angiogenesis is the propelling force for tumor growth of hepatocellular carcinoma (HCC), and antiangiogenic therapy may represents one of the most promising modalities for HCC treatment. The Akt pathway is activated by receptor tyrosine kinase growth factors that lead to the generation of membrane-bound phosphoinositides which then act as second messengers to activate Akt. Phosphorylation of Akt results in the activation of a cascade of different protein targets involved in apoptosis and cell proliferation. Recent data suggest that Akt pathway is involved in regulating tumor angiogenesis and metastasis. Little information exists regarding the role of Akt pathway in angiogenesis and metastasis of HCC.

Design: Formalin-fixed paraffin-embedded tissue sections of 29 HCC, 7 hepatocellular adenoma (HA), 27 cirrotic nodules (CN) and 8 normal liver tissues (NLT) were selected. Expression levels of phosphorylated forms of the constituent proteins (p-Akt, p-mTOR, and p-70 S6K) were evaluated by immunohistochemistry using standard avidin-biotin techniques. Endothelial marker, CD31 was used as positive control stain for endothelial cells. The number of immunoreactive endothelial cells of above reactants in sinusoidal endothelial cells was scored as follows: 0 (no immunoreactive cells), 1 (<10%), 2 (10–50%), and 3 (>50%). Staining intensity was graded as 0 (negative), 1+ (weak), 2+ (moderate), or 3+ (strong).

Results: The number of p-Akt and p-S6K positive sinusoidal endothelial cells and the intensity of immunostaining were significantly increased in HCC compared with HA, CN and NLT ($P < 0.01$). There was no significant correlation between the expression of p-Akt and p-S6K protein and the grade and stage of HCC. No significant difference of p-Akt and p-S6K expression was observed between HA and CN, and between HA and NLT. HCC exhibited immunoreactivity for p-mTOR, however, no significant difference in the expression was found between HCC and non-HCC groups.

Conclusions: Increased expression of phosphorylated proteins (p-Akt, and p-S6K) of Akt pathway in sinusoidal endothelial cells in HCC suggests that the Akt pathway may be involved in promoting tumor angiogenesis and metastasis. The findings may have significant implications in the development and application of targeted therapy. The molecular mechanism(s) of Akt pathway in HCC progression merits further investigation.

1409 Expression of α-Methylacyl Coenzyme A Racemase (AMACR/P504S) in Neoplastic and Nonneoplastic Liver Lesions

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Background: alpha-Methylacyl-CoA racemase (AMACR) is a mitochondrial and peroxisomal enzyme involved in the metabolism of branched-chain fatty acid and bile acid intermediates. AMACR, an immunomarker for prostatic adenocarcinoma, has been shown to be expressed in a variety of other neoplasms. This study aims to evaluate immunohistochemical expression of the AMACR in neoplastic and nonneoplastic liver lesions, and assess its value in the diagnosis of liver tumors.

Design: Formalin-fixed paraffin-embedded tissue sections of 32 hepatocellular carcinoma (HCC) (9 well, 14 moderately and 9 poorly differentiated), 7 hepatocellular adenoma, 28 cirrhotic nodules and 16 normal liver tissues were immunostained for AMACR using standard avidin-biotin techniques. The level of AMACR expression was categorized into four grades: 0 (negative), 1+ (weak), 2+ (moderate), or 3+ (strong) based on intensity of staining. The expression of AMACR was further assessed using two scales: high expression (2+ or 3+) and low expression (0 or 1+).

Results: High expression of AMACR was found in 85 % (27/32) of HCC tissue including 89 % (8/9) of well-differentiated HCC. In contrast, only 14 % (1/7) of hepatocellular adenoma, 15 % (4/26) of cirrhotic nodules and 6 % (1 /16) of normal liver tissues showed high expression for AMACR. The differences in AMACR expression between well-differentiated HCC and hepatocellular adenoma or cirrhotic nodules was significant ($P < 0.001$). Vascular invasion by HCC was observed more frequently in cases with AMACR high expression than those with AMACR low expression ($p<0.01$). No significant correlation was found between AMACR expression and HCC grade.

Conclusions: The expression of AMACR is highly enhanced in HCC. The results indicate that AMACR may be involved in hepatocarcinogenesis. AMACR staining may serve as a useful marker for the differential diagnosis of well-differentiated HCC from hepatocellular adenoma or cirrhotic nodules. Additional studies are needed to establish the role of AMACR as a biomarker of HCC.

1410 Expression of von Hipple-Lindau Gene Product (pVHL) and S100P in Cystic Neoplasms of the Pancreas – With an Implication for Their Roles in Tumorigenesis

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Background: S100P belongs to the family of S100 calcium binding proteins. Over-expression of S100P has been reported to be associated with tumor progression and a poor clinical outcome in some human carcinomas. The von Hipple-Lindau gene has been linked to the carcinogenesis of both hereditary and sporadic clear cell renal cell carcinomas. Our recent study demonstrated the nearly perfect inverse correlation of up regulation of S100P and down regulation of pVHL in pancreatic intraepithelial neoplasias (PanIN) and invasive ductal adenocarcinoma of the pancreas (Lin et al. AJSP 2007; in press). This study intends to investigate whether there is a correlation of expression of these two markers in common cystic neoplasms of the pancreas.

Design: We immunohistochemically evaluated the expression of S-100P and pVHL on conventional tissue sections of 97 cystic neoplasms of the pancreas. They included 23 cases of mucinous cystic neoplasm (MCN), 39 cases of intraductal papillary mucinous neoplasm (IPMN), 12 cases of solid-pseudopapillary tumor (SPT), and 23 cases of serous microcystic adenoma (SMA). The staining intensity and distribution were recorded.

Results: The results showed a nuclear and cytoplasmic staining pattern of S-100P in 21 of 23 MCN cases (91.3%) and 100% cases of IPMN. In contrast, none of the cases of SPT and SMA was positive for S100P. The 2 MCN cases with S100P negativity were positive for pVHL. All cases in MCN (except the 2 S100P negative cases), IPMN and SPT were negative for pVHL, including 20 MCN cases and 15 IPMN cases with low-grade dysplasia. In contrast, all SMA cases and adjacent normal pancreatic ducts were positive for pVHL.

Conclusions: Our data suggest 1) the findings of up regulation of S100P and down regulation of pVHL in MCN and IPMN are similar to that of PanIN and pancreatic ductal adenocarcinoma, indicating a role of these two proteins in early tumorigenesis; 2) the expression pattern of pVHL supports the recent recategorization of MCN and IPMN without any cytologic atypia as a low-grade dysplasia instead of an adenoma; 3) loss expression of pVHL in SPT supports the concept of an uncertain malignant potential of this entity; and 4) the finding of lack of expression of S100P and expression of pVHL in SMA supports the benignity of this entity.

1411 MicroRNA-21 Is Up-Regulated in Human Pancreas Cancer: A Tissue Microarray Study of 247 Cases Using In-Situ Hybridization

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Background: MicroRNAs (miRs) are small (~22nt) RNA fragments with diverse functions including regulation of cell differentiation and tumorigenesis. Differential expression of miRs in cancer may have diagnostic and prognostic value. Little data is available describing miRs distribution within cells or tissues. We have shown by RT-PCR and microarray chip analysis that several miRs are differentially up-regulated in human pancreatic adenocarcinoma (PA). One of the most highly overexpressed miRs was miR-21 (JAMA 297:1901-1908, 2007). The purpose of this study is to confirm overexpression of miR-21 in PA and determine cellular localization of staining in both malignant ducts and other cell types.

Design: 163 PA, 67 chronic pancreatitis (CP) and 17 normal pancreas (NP) were retrieved from archival files. Tissue cores from formalin-fixed, paraffin embedded donor blocks (2 cores per block) were arrayed to create a tissue microarray of cores measuring 2.0 mm each. Sections were cut at 5 μ, pre-hybridized with Exiqon's hybridization buffer. Exiqon DIG-labeled miRCURY LNA probes for miR-21, RNA U6 or Scramble controls were then added to slides at 50nM, hybridized for 20 hours, and detected using alkaline phosphatase conjugated anti-DIG Fab fragment (probes provided by Exiqon). Controls stained appropriately and slides were interpreted by 2 pathologists. MiR-21 was graded as negative, positive or strongly positive and nuclear and/or cytoplasmic localization were noted.

Results: Weak nuclear positivity was seen in lymphocytes, acini and islets in CP and NP. MiR-21 expression in ducts was seen in 117/163 (72%) PA, 12/67 (18%) CP and 1/17 (6%) NP (Table).

Comparison of miR-21 Expression

MiR-21 Expression	PA n=163	CP n=67	NP n=17
0 (negative)	46/163(28.2%)	55/67 (82.1%)	16/17 (94.1%)
1 (weak positive)	96/163(58.9%)	12/67 (17.9%)	1/17 (5.9%)
2 (strong positive)	21/163(12.9%)	0/67 (0%)	0/17 (0%)

PA= pancreatic adenocarcinoma, CP=chronic pancreatitis, NP=normal pancreas

None of the benign ducts showed strong expression. Of the 117 positive PA cases, strong expression was seen in 21 and weak expression in 96. MiR-21 predominantly stained nuclei with some cytoplasmic expression in PA.

Conclusions: We confirm miR-21 is overexpressed in ductal cells in PA as compared with CP and NP. Cellular localization is mainly nuclear but also cytoplasmic in some PA. MiR-21 appears to be involved in pancreatic carcinogenesis. Additional studies are warranted to further evaluate cellular localization of miR-21.

1412 Preoperative ALT Levels Are Associated with Centrilobular Fibrosis in Nonalcoholic Steatohepatitis (NASH) in Morbidly Obese Patients Undergoing Bariatric Surgery

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Background: Obesity-associated nonalcoholic liver disease (NAFLD) is increasingly recognized as a cause of cirrhosis. Our previous study showed resolution of NASH and regress of centrilobular fibrosis in morbidly obese subjects after the rapid weight loss from bariatric surgery. The aim of this study was to identify the variables associated with NASH (and fibrosis in NASH) in these histologically proven NASH cases.

Design: We retrospectively analyzed paired intra- and post-operative needle liver biopsies in 39 morbidly obese subjects following bariatric surgery according to the recent NIH-based histological scoring system for NAFLD. Centrilobular fibrosis were scored according to the relative length of fibrosis extending from the central veins (0: no fibrosis; 1, 2, and 3: centrilobular fibrosis extending to the inner 1/3 zone, the mid 1/3 zone, and the outer 1/3 zone, respectively). Advanced centrilobular fibrosis was defined as fibrosis extending to the mid 1/3 zone and beyond (≥ 2). The demographics and biochemical parameters were compared.

Results: Eleven patients (25.6%) had only steatosis, 24 patients (64.1%) had NASH, and the remaining 4 cases (10.3%) had normal liver histology on the initial intraoperative liver biopsies. The follow up liver biopsies performed at an interval of 18 months showed complete resolution of NASH in all NASH patients. The age, sex, preoperative BMI, preoperative fasting glucose levels and lipid profiles (HDL, LDL, triglyceride) were not different in NASH and non-NASH groups. Preoperative ALT and AST levels, however, were higher in patients with NASH in comparison to patients without NASH (ALT 37.8 U/L vs 20.5 U/L, p=0.0017; AST 31.0 U/L vs 19.5 U/L, p=0.0005). Furthermore, the preoperative ALT levels were higher in NASH cases with advanced centrilobular fibrosis than those with no or only mild centrilobular fibrosis (41.5 U/L vs 26.5 U/L, p=0.016). ALT levels were independent of other features (portal fibrosis, portal inflammation, lobular inflammation, or overall fibrosis) of NASH.

Conclusions: Our results suggest that elevated preoperative ALT levels are associated with NASH and advanced centrilobular fibrosis in morbidly obese subjects undergoing bariatric surgery.

1413 Global Histone Modifications as Markers of Pancreatic Ductal Adenocarcinoma Prognosis

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Background: Alterations in modifications of histones (acetylation and dimethylation) have been linked to deregulated expression of many genes with important roles in cancer development and progression. The purpose of this study was to investigate the expression pattern of acetylated and methylated histones and its prognostic value in pancreatic adenocarcinoma.

Design: 81 pancreatic ductal adenocarcinomas were retrieved from the Surgical Pathology archived files (year 1992-2006). Using whole sections, immunohistochemistry was performed with 5 commercially available anti-histone antibodies (H3K18Ac, Epitomics,1:50; H3K9Ac, Cell Signaling, 1:100; H4K12Ac, Abcam, 1:200; H3K4diMe, Abcam, 1:600 & H4R3diMe, Upstate, 1:50) on formalin-fixed, paraffin-embedded tissues. Staining intensity was scored "H" and "L" which defined the staining intensity in tumor was either higher or lower compared to normal parenchyma. The scores were analyzed statistically by CHI-TEST focusing on 4 pathological and clinical parameters including tumor size, grade, lymph node metastasis and survival.

Results: For the histone acetylation, 58% (26/45) of T2-4 carcinomas (tumor size >2cm) showed elevated intensity for H3K18Ac, while 69% (11/16) of T1 (≤2cm) showed lower intensity. 70% (19/27) T2-4 carcinoma displayed the pattern of H3 "H" scored with H4 "L" ($p<0.05$). Most of G2 (well and moderately differentiated) carcinomas exhibited lower intensity for H3K18Ac and H3K9Ac (60% and 85%, respectively). It was significant for "H" scored H3K9Ac in carcinoma with lymph node metastasis [65% (26/40 N1 carcinomas) compared to only 14% (3/22 N0 carcinomas), $p<0.001$]. For the histone methylation, methylated H4 expressed higher in T2-4 carcinomas (68%, 26/38) compared to T1 carcinomas (27%, 4/15) ($p<0.01$). It was also significant for the relationship between low intensity staining of methylated H4 and patient survival ($p<0.05$).

Conclusions: Our results demonstrate a distinct pattern of post-translationally modified histones in pancreatic ductal adenocarcinoma, indicating acetylated and methylated histones may serve as significant prognostic biomarkers for pancreatic carcinoma and implying it will be significant to further investigate its biologic role.

1414 High Frequency of Epidermal Growth Factor Receptor Expression in Pancreaticobiliary Adenocarcinomas

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Background: Pancreaticobiliary adenocarcinomas are highly aggressive malignancies with a dismal patient survival owning to limited availability of surgical and nonsurgical therapeutic modalities. A trend in cancer treatment in recent years is the development of various effective targeted therapies. One of the potential targets is epidermal growth factor receptor (EGFR), which has been shown to be expressed in a variety of malignancies. However, the expression status of EGFR in pancreaticobiliary adenocarcinomas has not been well investigated.

Design: A total of 34 pancreaticobiliary adenocarcinomas (22 pancreatic ductal adenocarcinomas and 12 cholangiocarcinomas) were included in this study. Formalin-fixed, paraffin-embedded tissue sections were immunohistochemically stained for EGFR expression utilizing the EGFR pharmDx kit (Dako) containing a mouse monoclonal anti-human EGFR antibody (clone 2-18C9). The immunostained slides were evaluated by two observers (MTM and HLW). Any membranous staining above background in tumor cells was considered positive according to the Dako EGFR pharmDx interpretation guide and the staining intensity was graded as weak (1+), moderate (2+) or strong (3+). Positive cases were also stratified according to the percentage of positively stained tumor cells into very focal (>0% to 5%), focal (>5% to 50%), and diffuse (>50%).

Results: A variable degree of EGFR expression was detected in 33 (97%) pancreaticobiliary adenocarcinomas (Table), among which 25 (76%) exhibited a diffuse 2+ to 3+ staining pattern and 8 (24%) showed focal immunoreactivity. The only negative case was a cholangiocarcinoma. None of the positive cases exhibited a very focal staining pattern.

Table. Immunohistochemical detection of EGFR expression in pancreaticobiliary adenocarcinomas

Tumor	3+/diffuse	3+/focal	2+/diffuse	2+/focal	1+/focal	Negative	Total
PDC	9 (26)	3 (9)	6 (18)	3 (9)	1 (3)	0	22
CC	7 (21)	0	3 (9)	1 (3)	0	1 (3)	12
Total	16 (47)	3 (9)	9 (27)	4 (12)	1 (3)	1 (3)	34

The numbers in parenthesesare % of cases based on the total number of 34. PDC, pancreatic ductal adenocarcinoma; CC, cholangiocarcinoma.

Conclusions: Pancreaticobiliary adenocarcinomas frequently express EGFR protein. These observations may have important therapeutic implications because EGFR-based targeted therapies have shown promise for other malignant neoplasms.

1415 Interobserver Variability in Evaluating Vascular Invasion in Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) is unique in that the presence of vascular invasion significantly changes tumor stage. Even though searching for vascular invasion is a common practice in surgical pathology, there appears to be a great variation among pathologists. The purpose of this study was to assess if HCC could be accurately staged using vascular invasion as a staging parameter.

Design: A total of 126 liver resections for HCC were included in this study. One to 3 representative H&E slides from each case were selected by one of the authors (MTM). Slides containing extrahepatic large vessel invasion, pure tumor without adjacent nonneoplastic liver tissue, or extensive necrosis secondary to tumor embolization were excluded from this study. The patient identifiers on the selected slides were removed and a random number was assigned to each slide. The slides were circulated among 6 practicing pathologists for independent review for small and large vessel invasion using their own criteria. The presence or absence of small and large vessel invasion on each slide was recorded as yes or no. The results were analyzed using unweighted kappa statistic analysis for multiple raters.

Results: A moderate interobserver agreement was achieved for small vessel invasion with a kappa value of 0.53 and a 95% confidence interval (CI) of 0.47-0.58. However, the kappa value dropped significantly to 0.18 (CI: 0.12-0.23) for large vessel invasion due to slight to fair interobserver agreement. If either small or large vessel invasion was considered, the overall interobserver agreement was no better than that for small vessel invasion only (kappa value: 0.52; CI: 0.46-0.57).

Conclusions: Though important for HCC staging, pathologists may have difficulty in interpreting vascular invasion in equivocal cases, leading to either understaging or overstaging of the tumors, which in turn may have significant impact on prognostic assessment and therapeutic decision making. Although the distinction between small and large intrahepatic blood vessels may not be critical for HCC staging, the need for improved definitions for these structures, especially for large vessels, is suggested by our observations.

1416 Ossifying Desmoplastic Nested Epithelial-Spindle Tumors of the Liver – A Clinicopathologic and Immunohistochemical Study of 8 Cases

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Background: Only 11documented cases of the distinctive ossifying nested stromal and epithelial tumor of the liver have been reported in the English literature.

Design: We reviewed clinical, histopathologic, and immunohistochemical features of 8 ossifying stromal-epithelial tumors arising in the liver from the AFIP files.

Results: These tumors occurred in 7 females and 1 male with mean and median ages of 20 and 18.5 years (range, 2-32 years). Four had a history of calcified hepatic nodules, believed to be calcified hemangiomas, since childhood (age 4-10). One patient had Cushing's syndrome that abated after excision. All tumors were discovered incidentally. Seven patients had a partial hepatectomy. One patient with an unresectable tumor underwent liver transplantation but died of postoperative complications. One had two local recurrences treated by radiofrequency ablation but was alive and well 14 years after excision. Three others were alive and well at 4 months, 2 years and 7 years after surgery. The tumors ranged from 5.5 to 20 cm (mean, 12.8 cm) and had a characteristic histologic appearance with irregular, sharply circumscribed nests and islands of bland appearing spindled to focally epithelioid cells, surrounded by a cellular desmoplastic stroma. Mitoses were present (1 to 5 per 10 HPF) in all cases as well as microscopic areas of extension of the tumor into the surrounding liver in 7 patients, but there was no vascular invasion. The tumor nests had focal psammoma-like calcifications in all cases with ossification in 4, dense osteoid-like collagen in another 3, and areas of myxoid degeneration or necrosis in 6 cases. Immunohistochemistry in 7 cases showed

at least focal positivity for cytokeratin (clones AE1/AE3). The nests were also positive for vimentin (5/5) and NSE (3/3), and S-100 protein was positive in one case (1/7). All tumors tested were negative for cytokeratin 7 (5/5), cytokeratin 20 (5/5), chromogranin (6/6), synaptophysin (7/7), Leu 7 (4/4), HMB45 (3/3), Desmin (4/4), inhibin (3/3), CD117 (2/2), CEA (3/3) and HepPar-1 (3/3) and alpha-fetoprotein (5/5). The cells of the stoma surrounding the nests appeared to be fibroblasts and myofibroblasts and expressed SMA (6/6).
Conclusions: Ossifying nested stromal and epithelial tumors of the liver are a rare clinicopathologic entity of uncertain histogenesis. Based on currently available information, this tumor is best considered a low grade malignancy.

1417 Validation of a Feature Recording System for Hepatic Graft-Versus-Host Disease

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Background: The histologic assessment of hepatic graft-versus-host disease (GVHD) has many unresolved issues and practical difficulties. The Pathology Working Group of the NIH Consensus Development Project on Criteria for Clinical Trials in Chronic GVHD has published a feature recording system for GVHD assessment in different organs. We undertook a pilot study to validate the system for hepatic GVHD.
Design: 35 biopsies done to rule out hepatic GVHD from 1999 to 2006 were evaluated using the proposed GVHD feature recording system. Laboratory data at biopsy including ALT, AST, alkaline phosphatase and total bilirubin levels were used to classify liver injury as hepatitic, cholestatic or mixed hepatitic/cholestatic based on standard classification used in drug-induced liver injury. GVHD scores were compared with laboratory parameters.
Results: There were 23 males and 10 females with a mean age of 36 years (range 9 - 65 years), who had received bone marrow/stem cell transplantation for hematolymphoid disorders, solid tumors or chronic granulomatous disease. Of the 35 biopsies, 21 were classified as 'GVHD', 6 as 'Consistent with GVHD', 7 as 'Possible GVHD' and 1 as 'Not GVHD'. Bile duct changes included ductitis (28/35, 80%), epithelial dysmorphism (31/35, 89%) and ductular proliferation (26/35, 74%). Two (6%) cases had significant ductopenia. Significant portal inflammation was present in 10 (29%) cases, interface hepatitis in 13 (37%) cases, lobular inflammation in 12 (34%) cases and central necrosis in 12 (34%) cases. Portal fibrous expansion and/or septal fibrosis was seen in 16 (45%) cases and sinusoidal fibrosis in 4 (11%) cases. Based on biochemical parameters, 16 (45%) cases were classified as cholestatic, 10 (29%) as hepatitic and 9 (26%) as mixed. Total inflammatory scores were significantly higher in the hepatitic group than in the cholestatic group. AST levels were significantly higher in cases with moderate to severe interface hepatitis. Alkaline phosphatase levels showed significant correlation with severity of ductitis and epithelial dysmorphism, while degree of cholestasis correlated with total bilirubin levels. Additional histologic findings were steatohepatitis (3/35, 9%), iron overload (32/35, 91%) and venoocclusive disease (1/35, 3%).
Conclusions: The proposed hepatic GVHD feature recording system shows promising correlations with biochemical parameters and provides a sound semiquantitative assessment of liver biopsies following marrow/stem cell transplantation. Biopsies also have the potential to reveal other significant disease that may contribute to hepatic dysfunction.

1418 Cytochrome *c* Oxidase Subunit I Deficient Foci in the Liver: A Distinct and Novel Surrogate Biomarker of Early Hepatocarcinogenesis

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Background: Cytochrome oxidase *c* subunit I (COX I) is a key mitochondrial enzyme involved in apoptosis regulation. Aberrant expression of COX I has been identified in colon cancer and is associated with oxidative stress-induced gene mutations. Our study explored the potential of COX I as an early surrogate biomarker of liver pathogenesis by comparing COX I expression patterns in hepatitis C (HCV) cirrhosis and hepatocellular carcinoma (HCC) to that of normal control liver.
Design: Explants of cirrhotic liver, with and without HCC, and segmental resections of HCC adjacent to non-cirrhotic liver were selected for this study. Sixteen cases of cirrhosis with HCC, 16 cases of HCC arising in non-cirrhotic liver, 18 cases of HCV cirrhosis, and 3 control liver sections with no pathological change were identified. Immunohistochemistry was performed for COX I on representative paraffin blocks from each case. Normal liver tissue served as a positive control and also contained internal negative controls.
Results: Extensive positive staining for COX I was seen in hepatocytes and bile duct epithelial cells of normal liver (Fig. A). Cases of HCV cirrhosis showed staining loss in multiple foci (focus defined as ≥25 hepatocytes; mean total area of foci=0.68cm^2, mean number=0.38 foci/cm^2 +/- 0.45 (Fig B)). A marked decrease in number and size of foci was seen in non-cirrhotic liver (mean total area=0 .15 cm^2, mean number=0.09 foci/cm^2 +/- 0.17 (Fig.C)). Six of 16 cases of HCC, both with and without coexisting HCV, showed extensive loss of COX I (Fig. D) while the remainder showed focal or no loss of expression.

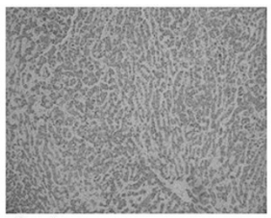
Figure A

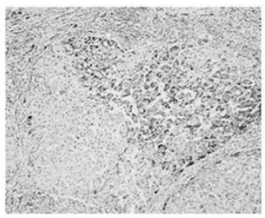
Figure B

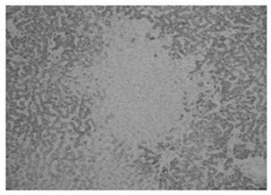
Figure C

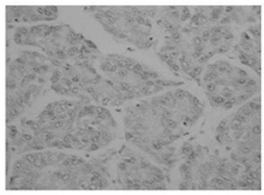
Figure D

Conclusions: Frequent foci of absent COX I expression were seen in HCV cirrhosis and liver parenchyma adjacent to HCC, but not in normal liver. In addition, more COX I-deficient foci were found in cirrhotic liver than in non-cirrhotic liver adjacent to HCC, implying that chronic hepatitis/cirrhosis may be a major cause for lost COX I expression. Our results indicate that loss of expression of COX I may serve as a useful biomarker for hepatocarcinogenesis and may also represent a potential surrogate marker for use in the detection and prevention of HCC in patients with HCV cirrhosis.

1419 Under-Diagnosis of Chronic Hepatic Allograft Rejection in Routine Needle Biopsies Is More Due to Non-Recognition of Ductopenia Than Inadequate Sampling

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Background: Histological diagnosis of chronic rejection (CR) on routine needle biopsies of hepatic allografts is considered challenging due to the need for sufficient numbers of portal areas to ensure loss of 50% of interlobular bile ducts and adequate representation of medium-sized artery branches to assess for obliterative arteriopathy (OA). We compared routinely performed transcutaneous and transvenous needle core biopsies in native livers and grafts with CR to assess sample adequacy in CR. We also evaluated failed explanted grafts with CR for OA, and the role of OA in sample adequacy of needle biopsies in CR.
Design: From our files we selected 10 patients with explanted hepatic allografts failed due to CR who had prior transcutaneous or transvenous needle biopsies with the diagnosis of CR. Additional 20 controls of age-matched patients with needle biopsies (10 transcutaneous and 10 transvenous) performed by the same operators (surgeons and interventional radiologists) were selected from non-transplanted livers for comparison. The CR needle biopsies and the control cases were reviewed for sample size comparisons, numbers of portal areas and binary arterio-venous versus solitary venous channels to distinguish portal versus centrilobular regions. The explanted CR grafts were reviewed to assess presence and frequency of OA and the likelihood of recovering the lesion in needle core biopsies.
Results: There were no statistically significant differences in sample size (mean combined total length of 1.5 cm and diameter of 0.2 cm) between the CR and control needle biopsies, both transcutaneous and transvenous. When binary arterio-venous channels were used as portal markers, the numbers of portal tracts (mean of 17) were also statistically similar between the two populations. The failed grafts showed OA in 7/10 cases and typically in only 1-2 of 7 protocol sections from peri-hilar areas and none from peripheral areas.
Conclusions: Routinely performed transcutaneous or transvenous needle biopsies by experienced operators on hepatic allografts are adequate for assessment of ductopenia in CR. Portal areas in CR tend to mimic centrilobular areas and may be unrecognized due to depleted portal inflammatory cells and ductopenia. When portal tracts are identified by 'paired' portal vein and hepatic artery branches, ductopenia is more easily appreciated. OA is quite focal and peri-hilar in explanted CR grafts, and its recovery in needle biopsies should not be expected or used for assessing sample adequacy.

1420 Positive Predictive Value of Diagnosing Chronic Rejection on Needle Biopsies before Hepatic Allograft Failure

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Background: Histological recognition of chronic rejection (CR) in needle biopsies from hepatic allografts is crucial both for salvaging the graft in the early stage of CR and planning for re-transplantation in failing grafts. However, limitations such as sample adequacy, confirmation of ductopenia/duct atrophy and lack of recovery of medium-sized arteries are well documented challenges in graft needle biopsies. We performed a retrospective study to evaluate the predictive value of diagnosing CR on routine graft needle biopsies before the failed grafts were removed and the patients re-transplanted.
Design: Between 2003 and March of 2007, 419 liver transplants were performed at our institution. Among these were 14 failed grafts due to chronic allograft rejection (8 M, 6 F, 29-65 yrs, mean 48). The following were reviewed: interval between the two transplants, number of pre-failure needle biopsies and their diagnoses, interval between the last graft biopsy and graft failure, prior episodes of acute cellular rejection, and explanted failed grafts. We also investigated if any patient with graft needle biopsy diagnosis of CR had a clinical outcome other than CR of the graft (graft failure, death, rejection reversed by treatment).

Results: 10/14 (71%) of explanted failed grafts had a clear diagnosis of CR on prior graft needle biopsies, with a median interval of 10 months between intial transplant and graft failure, mean of 4.6 biopsies and median of 20 days from last biopsy to re-transplant. 4/14 (29%) patients who had no evidence of CR on pre-failure graft biopsies had fewer biopsies (mean 3.0) and a median of 63 days from last biopsy to re-transplant. In both groups, about 80% had prior episodes of acute cellular rejection. 9/10 graft needle biopsies with diagnoses of CR resulted in graft failure due to CR, indicating that a diagnosis of CR on needle biopsy has a 90% positive predictive value.
Conclusions: Inspite of well-acknowledged challenges and limitations, routine needle biopsies from the graft provide a high (90%) positive predictive value with a sensitivity of 71% for diagnosing CR. Thus, the purported under-diagnosis of CR is more due to clinical factors, such as less frequent biopsies and delay in recognition of graft dysfunction rather than the histological assessment of graft needle biopsies.

1421 Unexpected Cirrhosis and Vascular Findings in the Gastric Bypass Population: Implications for Pre-Operative Screening

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Background: Morbid obesity is associated with non-alcoholic fatty liver disease (NAFLD); this liver disease may be variably associated with progression to cirrhosis. Because gastric bypass is an invasive therapeutic procedure, pre-operative assessment of co-morbidities is important. The goal of our study was to assess the liver biopsy findings at the time of gastric bypass.
Design: Liver biopsies obtained on a routine basis from 63 morbidly obese patients at the time of surgery were scored for the degree of steatosis, steatohepatitis, portal and space of Disse fibrosis (Trichrome stain). Portal fibrosis was scored on a 0-4 (cirrhosis) scale while steatosis and steatohepatitis were scored on a 0-3 scale. Other incidental histologic findings were also noted.
Results: Unexpectedly, at the time of gastric bypass, 14% of patients had cirrhosis, and an additional 6% had portal tract venopathy with nodular regenerative hyperplasia. Steatosis was present variably (grade (%patients): 0 (6%), 0.5 (11%), 1 (19%), 2 (46%), 3 (18%), as was steatohepatitis (0 (32%),1 (50%), 2 (16%), 3 (2%)). With the exception of those with cirrhosis, the extent of portal fibrosis was relatively low (0 (63%), 1 (13%), 2 (7%), 3 (3%)). In keeping with NAFLD, space of Disse fibrosis was variably present (0 (84%), 0.5 (3%), 1 (5%), 2 (8%), 3 (0%)).
Conclusions: Two significant and separate entities, cirrhosis and portal tract venopathy with nodular regenerative hyperplasia, were clinically unexpected findings in 14% and 6% respectively, of morbidly obese patients undergoing gastric bypass. Because of their association with decreased liver function and/or portal hypertension, pre-operative diagnosis of these entities may be important. Rare (6%) morbidly obese patients do not have NAFLD. Routine liver biopsy for complete evaluation is recommended at the time of surgery, as in our population 20% of patients had significant unexpected liver findings.

1422 Autoimmune Pancreatitis: West vs. East

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Background: "Autoimmune pancreatitis" (AIP) appears to have a heterogenous morphology. Growing evidence led to an impression that AIP seen in the west may be different from that in the east. Thus, we reviewed and compared resected AIP cases of the US with those of Japan.
Design: The study group consisted of 53 US AIP (US-AIP) and 38 Japanese AIP (J-AIP) cases. In each case, two observers evaluated histologic features characteristic of AIP including overall histologic pattern (lymphoplasmacytic sclerosing pancreatitis [LPSP] vs. idiopathic ductocentric chronic pancreatitis [IDCP]), the pattern of pancreatic involvement by inflammation (ductocentric vs. lobulocentric) and the extent of inflammation in interlobular and intralobular ducts, lobules and fibrosis, reactive epithelium of ducts, inflammatory pseudotumor-like changes, obliterative phlebitis and panniculitis. IgG4 stain was performed and an average number of IgG4+ plasma cells/HPF (IgG4 PC) was recorded. The demographic data and histologic features were compared between the 2 groups.
Results: US-AIPs were significantly younger than J-AIPs (mean age: 56 years vs. 63 years, $p<0.05$) and more female patients were seen in the US than in Japan (18/53 vs. 7/38). IDCP was documented frequently in US-AIPs compared to J-AIPs (23/53 vs. 7/38, $p<0.05$); however, there was no difference in the pattern of involvement between the 2 groups. Among individual histologic features, neutrophilic infiltrate in interlobular ducts, intralobular ducts and lobules and reactive epithelium of interlobular ducts were more extensive in US-AIPs than in J-AIPs ($p<0.01$, $p<0.05$, $p<0.05$ and $p=0.06$, respectively). Conversely, J-AIPs exhibited more prominent lymphoplasmacytic infiltrate in both lobules and fibrosis than US-AIPs ($p<0.001$). IgG4 PC was greater in J-AIPs than in US-AIPs (mean 78.5 vs. 41.1, $p < 0.05$). However, when only LPSP cases were compared, there was no difference in clinicopathologic features between the 2 groups.
Conclusions: The resected US-AIPs and J-AIPs appear to have different clinicopathologic characteristics; however, they could be summarized as the difference in the prevalence of IDCP between the 2 groups.

1423 Regressing Lymphoplasmacytic Sclerosing Pancreatitis (LPSP)

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Background: LPSP is a histological prototype of autoimmune pancreatitis (AIP) in which a fibrosing process with lymphoplasmacytic infiltration involves the pancreatic parenchyma, peripancreatic soft tissue and veins. Although the spontaneous regression of AIP has been reported, little is known about LPSP in the course of regression. Thus, we reviewed a large cohort of resected AIP cases to identify histologic features of regressing LPSP.
Design: The study group consisted of 91 patients with a post-operative diagnosis of LPSP or idiopathic ductocentric chronic pancreatitis. All the H&E slides of resected pancreata were reviewed. Elastic stain and IgG4 immunostain were also performed.
Results: Although various degrees of regression were observed in LPSPs, 5 cases at the extreme end were selected for the further study. The overall histological features of these cases resembled nonspecific chronic pancreatitis except for the presence of dense periductal inflammation. Pancreatic lobules were destroyed and replaced by dense fibrosis with occasional storiform formations. Although the fibrosis appeared paucicellular, lymphocytes and plasma cells could be found on a careful examination. Periductal lymphoplasmacytic infiltrate was out of proportion to lobular inflammation; however, neutrophilic infiltrate or reactive changes of the ductal epithelium were none or rare. Elastic stain revealed fibrosing obliteration of medium-sized veins without lymphoplasmacytic phlebitis. Fibrosis was also prominent in the peripancreatic tissue without remarkable inflammation. However, numerous lymphoid follicles with germinal centers were noted in 3 cases and IgG4 immunohistochemistry revealed patchy aggregates of positive plasma cells in each case. The clinical features of these patients were similar to those of typical LPSP cases except that 2 of the 5 patients were asymptomatic at presentation.
Conclusions: The unique morphology characterized by periductal lymphoplasmacytic infiltrate, fibrous obliteration of medium-sized veins and numerous lymphoid follicles seen in the background of nonspecific fibrosis of the pancreatic parenchyma most likely represents regressing LPSP. Pathologists need to be aware that LPSP may be confused with nonspecific chronic pancreatitis, and thorough histologic evaluation as well as appropriate clinical correlation is warranted.

1424 Whipple Resections in Patients without Tumor: What Are Potential Mimickers?

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Background: Some Whipple resections performed for a clinical suspicion for malignancy revealed no evidence of malignant or benign tumor on pathologic examination. We classified diseases that mimicked malignant tumor clinically in a large series of Whipple resections.
Design: 461 Whipple resections were retrieved from our surgical pathology files during 1993-2007. 45 cases (9.7%) revealed no neoplastic disease, of which 35 cases were performed for a clinical suspicion of malignancy. Clinical information was collected and H&E slides were reviewed to classify the diseases.
Results: The mean age of patient is 55.9 years (range 22-87), and there was a male predominance with 29 males (64.4%) and 16 female (35.6%). The cause of suspicion for malignancy was radiologic evidence of a pancreatic mass in 21 patients (60%), periampullary tumor in 3 patients (8.6%), bile duct stricture or mass in 10 patients (28.6%) and positive or suspicious cytology in 3 patients. (8.6%). The classification of diseases is shown in the table below.

Classification of disease in 45 non-neoplastic Whipple resections

	No. of patients (%)	Clinically Suspicious (%)
Chronic Pancreatitis	**33 (73.3%)**	**23 (69.7%)**
Alcohol-associated	11(24.4%)	7 (63.6%)
Gallstone-associated	1 (2.2%)	1 (100%)
Autoimmune	10 (22.2%)	10 (100%)
Familial	1 (2.2%)	0 (0%)
Pancreas divium-associated	1 (2.2%)	1 (100%)
Unknown etiology	9 (20.0%)	5 (55.6%)
Biliary tract disease	**10 (22.2%)**	**10 (100%)**
Primary sclerosing cholangitis	2 (4.4%)	2 (100%)
Isolated common bile duct stricture	5 (11.1%)	5 (100%)
Choledocholithiasis	2 (4.4%)	2 (100%)
Ampulla of Vater ulcer	1 (2.2%)	1 (100%)
Duodenal Disease	**1 (2.2%)**	**1 (100%)**
Duodenal ulcer	1 (2.2%)	1 (100%)
Other	**1 (2.2%)**	**1 (100%)**

Reexamination of the slides revealed that 7 cases formerly diagnosed with chronic pancreatitis NOS are classified as autoimmune pancreatitis. The staging of 7 alcohol-associated pancreatitis mimicked malignancy are Stage I in 2 cases, II in 3 cases, III in 1 case and IV in 1 case.
Conclusions: Autoimmune pancreatitis is not uncommon disease in the U.S., and accounts for the largest portion of Whipple resections in the patients without tumor. Alcohol-associated pancreatitis accounts for the second largest, and early stage of alcohol-associated pancreatitis mimicked malignancy more frequently than late stage.

1425 Evaluation of Expression and Activation Status of NF-*k*B in Pancreatic Ductal Carcinoma

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Background: Pancreatic cancer is now one of the most common causes of cancer death worldwide. K-ras mutations are present in up to 90% of pancreatic cancer cases. The

expression of mutant K-ras activates the Akt/protein kinase B pathway, resulting in the activation of the nuclear factor-kappaB (NF-*k*B) transcriptional factor. Based on in vitro studies, constitutive NF-*k*B activity plays a key role in pancreatic carcinoma. In this study we investigated the expression of the p65 subunit of the NF-kB complex. In addition we investigated the activation status of NF-*k*B by phospho-I*k*Balpha antibody.
Design: A tissue microarray based on material obtained from 102 patients followed up at Harper University Hospital, Karmanos Cancer Center were utilized. The antibody staining was scored by combining staining intensity with percentage of tumor staining. The antibodies used were NF-kB p65 and phospho-IkBalpha(ser32/36), both from Cell Signaling Technology. The staining scores were correlated with margin and lymph node status, stage, tumor size, survival, Her2, DPC4, p53, and Kras mutation status.
Results: The staining was nuclear (p65) and cytoplasmic (p-I*k*Balpha) respectively. In normal pancreas there was weak staining in the ducts, and focally in the acini, whereas in areas of chronic pancreatitis there was more intense staining. In general there was an increased expression and activation of NF-kB in the carcinomas, compared to non-tumoral regions. None of the markers had a significant correlation with the overall survival. NF-*k*B(p65) expression had a correlation with lymph node status (p=0.04). All the other parameters tested, had no correlation with NF-*k*B(p65) or p*Ik*Balpha.
Conclusions: NF-kB expression or NF-kB activation do not have direct effects on the overall survival of pancreatic ductal carcinoma. The correlation with the lymph node status suggests a role in invasive properties of the tumor. Activation of NF-kB is most likely an early event in pancreatic carcinogenesis. Despite the lack of an effect on overal survival, due to its increased activation in pancreatic cancer, NF-*k*B is still a good target for therapeutic interventions.

1426 Delayed Veno-Occlusive Disease after Busulfan/Fludarabine Conditioning for Allogeneic Stem Cell Transplantation

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Background: Veno-occlusive disease (VOD) is a complication of conditioning chemotherapy for stem cell transplantation. VOD normally affects the liver within 30 d due to endothelial damage caused by chemotherapeutic agents, particularly cyclophosphamide. Recently IV fludarabine and busulphan was proposed as an alternative to standard chemotherapy with reduced hepatotoxic effects. In an initial report only 2 of 96 patients developed VOD (de Lima, M. et al. 2004. Blood. 104:857). One had been previously treated with cyclophosphamide and the other ingested large doses of acetaminophen. In contrast to this initial study, we report 7 cases of VOD among 34 patients treated with a similar regimen.
Design: Liver specimens (5 biopsies and 1 autopsy) and clinical data from 7 patients treated with IV fludarabine, busulphan, and alemtuzumab with a diagnosis of VOD were analyzed.
Results: Clinical features: All patients participated in a prospective study of conditioning with fludarabine, IV busulfan, and alemtuzumab. Busulfan was targeted to an AUC of 4800-6800 minumol/L. GVHD prophylaxis consisted of tacrolimus. All had advanced hematologic malignancies. These patients developed liver dysfunction and ascites 34-78 d (median 57 d post transplant). Liver biopsies confirmed VOD in 6 of 7 patients. Two patients had moderate VOD and recovered. One responded to defibrotide, but has residual portal hypertension. Four patients died from VOD and its complications. Histologic features: All 6 cases demonstrated centrilobular congestion and hemorrhage. Reticulin stains highlighted deposition of reticulin fibers wthin the centrilobular hepatic sinusoids and terminal hepatic vein lumens. In 4 of 6 patients zone 3 hepatocyte necrosis was also evident.
Conclusions: We report 7 cases of VOD resulting from treatment with IV fludarabine, busulphan, and alemtuzumab for patients with advanced hematologic malignancies. In contrast to traditional VOD, a syndrome of late-onset VOD was observed (mean = 57 d). In summary, VOD does occur in patients treated with high dose busulfan and fludaribine containing regimens, despite the absence of cyclophosphamide and post-transplant methotrexate. Its occurence is delayed compared with traditional VOD.

1427 Hepatolithiasis in Explanted Livers: Increasing the Spectrum of Disease

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Background: Hepatolithiasis or intrahepatic calculi are prevalent in East Asia, but occurs much less frequently in Western countries. In the East it is regarded as a separate entity altogether, the majority of which are associated with recurrent pyogenic cholangitis with or without parasitic infestations ("Oriental cholangiohepatitis"). However, apart from immigrant population from endemic countries, hepatolithiasis in the Western world is very rare and generally thought to be secondary to stones originating in the gall bladder or primarily resulting from benign strictures, sclerosing cholangitis, choledochal cysts or malignant biliary tumors. Hepatolithiasis, is also know to occur with biliary casts, a rare complication in orthotopic liver transplant (OLT) recipients mainly secondary to ischemia. The aim of this study was to report our experience of hepatolithiasis in explanted native livers as well as failed grafts in a tertiary care center.
Design: Between 2002 and 2007, among 610 explants, 18 adult patients with diagnosis of hepatolithiasis were identified in our pathology databases. The diagnosis of hepatolithiasis was based on macroscopic and histological detection of diffuse intrahepatic stones in explanted native livers and failed grafts. The following additonal information was collected: age and sex of patient, native liver disease, cause of graft failure, clinical presentation, and radiographic results.
Results: Eighteen patients (10 female, 7 male; mean age 51, range 45-68 years) with hepatolithiasis were included in this retrospective study. 10/18 cases of hepatolithiasis were diagnosed in OLT patients who had graft failure due to biliary cast disease. The remaining 8 cases were from native explanted livers. The causes of liver disease in the native explanted livers included the following: Alagille's (1), Oriental cholangiohepatitis (1), primary sclerosing cholangitis (3), primary biliary cirrhosis (PBC) (1) and autoimmune hepatitis (AIH) (1). 12/18 cases had associated radiologic findings such as dilated intrahepatic bile ducts and/or stones.
Conclusions: The majority of cases of hepatolithiasis in the Western world occur in combination with biliary casts in failed grafts. In the native livers, hepatolithiasis is quite rare and associated with diseases not hitherto linked with hepatolithiasis such as Allagille's, PBC, PSC and AIH, but having bile duct injury as a vital component. Given the spectrum of associated conditions, hepatolithiasis is best regarded not as a specific disease but a rare complication of diverse biliary diseases.

1428 Hepatitis C (HCV): A VAMC Pathology Report

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Background: HCV is a major blood-borne cause of chronic liver disease with severe complications including cirrhosis and hepatocellular carcinoma. Former military personnel are a high risk group for HCV with unique characteristics including co-morbidities and an ethnic distribution that may affect the response to therapy. Response to the numerous anti-HCV regimens is being evaluated in an ongoing study involving 24 VA medical centers. This response appears to correlate with many factors including but not limited to ethnicity, HCV genotype (GT) and other associated risks (i.e. alcohol, HIV, patients social activities, behavior etc.) We report our pathologic findings including a comparison of ethnic distribution of liver damage with the 3 HCV GTs.
Design: Between 2000-2006, HCV positive patients among 1050 screened veterans (immunoassay) were referred to HCV clinic for further care (including morphologic assessment). 251/1050 subjects showed HCV RNA in peripheral blood (quantitative PCR). Two investigators reviewed 249 liver biopsies to establish the staging and grading of liver damage (Scheuer modification of Knodell scoring system on H&E, trichrome, iron and reticulin.) χ^2 was used for statistical analyses.
Results: The subject population was >95% male with ethnicity approximately 60% Caucasian (C) and 40% African-American (AA). The general ethnic distribution at the facility is 70% C and 30% AA indicating an ethnic preponderance for HCV infections in AA males (p<0.0005). GT 1 was more prevalent in AA (87.1% vs 64.2%; p<0.05).

Table 1. Quantitative HCV RNA

	C	AA	
GT1	64.2%	87.1%	NS
GT2	26.6%	12.9%	NS
GT3	9.4%	0%	<0.0004

NS=not significant

Comparing GT 1 in C to AA, the prevalence of mild- moderate injury was not significantly different. However, severe injury (established cirrhosis; stage 3.5-4.0) was more prevalent in C vs AA (44% vs 9.3%; p<0.05).

Table 2. GT1 and liver injury

Severity	C	AA
Very mild (0.5-0.9)	0.8% (1)	0% (0)
Mild (1-1.9)	18.7% (23)	26.7% (20)
Mod. (2-2.5)	56.9% (70)	53.3% (40)
Incomplete Cirrhosis (3-3.4)	14.6% (18)	20% (15)
Established Cirrhosis (3.5-4)	43.9% (54)	9.3% (7)

Conclusions: 1. An ethnic preponderance for HCV infection in AA males and a greater prevalence of GT1 in AA compared to C, in our patient population. 2. High prevalence of established cirrhosis with GT 1 in AA as compared to C. This difference is not seen in GT 2 or 3. 3. Further investigation of individual co-morbidities that potentiate damage from GT 1, stratified by ethnicity is warranted.

1429 Cellular Prion Protein Associated with High Expression of P53 in Pancreatic Adenocarcinoma Correlates with Poor Prognosis

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Background: Cellular prior protein has been detected in many normal cell types but not pancreatic ducts. The normal cellular prion protein (PrP^C) is a glycosyl-phosphatidylinositol anchored membrane protein. The functions of PrP^C are cell type dependent. Depending on the cell type, it may participate in cell signaling, function as a metal transporter, a cell adhesion molecule, or regulate apoptosis activities. In our earlier study, we found PrP^C to be over-expressed in 46% of pancreatic carcinoma which may be associated with prognosis. In this study, we wanted to explore the relationship between P53 and PrP^C status in pancreatic adenocarcinomas, and investigate the possible combined role of P53 and PrP^C in pancreatic carcinogenesis and prognosis.
Design: Eighty-three consecutive cases of primary pancreatic carcinomas were selected. Sixty three of these 83 carcinoma cases had regional lymph node metastasis. Immunohistochemical study was performed on tissue microarray slides using monoclonal antibodies specific for PrP^C and P53 respectively. Clinical follow-up up to 5 years were recorded in 37 patients. Patients' ages range from 30 to 81 years old.
Results: By immunohistochemistry, PrP^C and P53 proteins were not detected in normal acinar, small and large non-neoplastic ductal epithelium in all cases. We found that PrP^C was over-expressed in 41% (34/83) of pancreatic carcinomas, and P53 was over-expressed in 39% (32/83) of pancreatic carcinoma. The expression of P53 was 65% (22/34) in PrP^C over-expressed cancers, which was much higher than cancers with no PrP^C expression, where 29% (10/34) expressed P53 (P<0.01). Nevertheless, the co-expression of PrP^C and P53 correlated with the poorest prognosis as compared with P53 or PrP^C alone or none, as shown in the table.

PrPC	P53	No. of cases	Mean Survival	3 yr survival rate
-	-	16	875 days	31%
-	+	6	942 days	33%
+	-	4	473 days	0
+	+	11	317 days	0

Conclusions: Our data indicate that the PrP^C protein was over-expressed in a group of pancreatic carcinoma and highly associated with P53 expression. Since PrP^C could function as an apoptosis regulator in vitro cell culture study, it might play an important role in the carcinogenesis of a subset of pancreatic carcinoma by P53 mediated apoptosis. The co-expression of PrP^C and P53 in the tumor correlates with a very poor prognosis in pancreatic carcinomas.

1430 Microvascular Density Image Analysis Using CD34 Staining Reliably Differentiate Well-Differentiated Hepatocellular Carcinoma from Hepatocellular Adenoma

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Background: The histologic differentiation of well-differentiated hepatocellular carcinoma (WDHCC) from hepatocellular adenoma (HA) can be challenging, especially when evaluating small needle core biopsy specimens. In the case of WDHCC arising in non-cirrhotic liver or HA occurring outside the usual clinical context of young female, with oral contraceptive use, the distinction can further be blurry. Capillarization of sinusoids resulting in CD34 positivity is a feature shared by both WDHCC and HA. We hypothesized that the microvascular density (MVD) resulting from such capillarization differs between WDHCC and HA, reflecting a difference in the ratio of sinusoids-to-cells making up the hepatic plates. This would imply that the thicker the plates, the fewer the interspersed sinusoids per surface area, translating to lower overall MVD. In this study therefore we have utilized an image analysis technique to objectively measure the differences in MVD between these two tumours.

Design: Surgically-resected, paraffin-fixed, formalin-embedded WDHCC in the context of cirrhosis (4 cases) and typical HA (3 cases) were identified from the records of the University Health Network, department of Pathology. CD34 stains were performed on representative sections in all cases. MVD analysis was performed using the DACO **ACIS®** automated cellular imaging system in conjunction with micro-vessel density application software. Eighteen 20x fields were evaluated from each tumor, and results evaluated for within-group differences using one-way ANOVA, generating an F statistic. Pooled data from each group were then compared using the t-statistic.

Results: There was no significant variation in MVD from one tumor area to another. There was no statistically significant differences in MVD within each group (WDHCC *$p=0.0108$;* HA *$p=0.212$*). There was however a significant difference in the microvascular density between WDHCC and HA, with latter having higher density (mean=36.17; CI (30.54, 40.81) as compared to WDHCC (mean=11.79; CI (9.77, 13.81)).

Conclusions: Significant differences exist between WDHCC and HA MVD to the extent that this parameter could be useful in resolving diagnostic dilemmas in borderline and difficult cases, and has the potential for use in small needle biopsies given the fairly uniform pattern of MVD.

1431 Atypical Neonatal Obstructive Liver Disease (NOLD): Variants of Biliary Atresia (BA) or Not?

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Background: Neonates with BA typically have a biliary obstructive histologic pattern that presents at 4-7 wks of age and progresses to biliary cirrhosis; an atretic or absent gallbladder (GB) can help establish the diagnosis. We describe 6 cases of NOLD (also known as neonatal or perinatal sclerosing cholangitis) with early clinical or pathologic findings atypical for BA to determine if these cases could represent variants of BA or remain a distinct entity.

Design: 6 cases of NOLD were compared to 6 cases of typical BA. This included clinical features including gestational age and congenital anomalies; lab, operative and radiographic results; and grading of end-stage liver samples in a semiquantitive manner for ductal plate malformation, degree and patterns of fibrosis, ductular and lobular inflammation and bile stasis, bile infarcts, ductopenia, hepatocyte acinar or giant cell change, and vascular changes. Cases were stained with copper, trichrome, EVG, CD56, CK7, and D240. A diagnosis of a metabolic disease and/or paucity of duct syndrome excluded the case.

Results: In all cases, patterns of fibrosis and ductal/ductular changes (including degree of ductopenia, periduct fibrosis, large duct sclerosis) were similar; increased hepatic arteries, prominent portal/hilar lymphatics, prominent central vein sclerosis, and minimal copper deposits were noted. Mean and median age at diagnosis of BA was 9 wks, range 4-14. Mean and median age of histologic diagnosis of NOLD was 23 wks, range 6-38. In NOLD cases, 2 had features more typical of neonatal hepatitis at 5 and 8 wks, 2 had jejunal atresia with transient TPN use (one of which had septo-optic dysplasia). 5 cases were transplanted in each category, with mean age for BA 37 wks, median 24, range 17-93 and mean age for NOLD 46 wks, median 48 wks, range 40-52. By ultrasound, NOLD patients had GBs; but in BA, GBs were absent or atretic (confirmed at surgery). At explant, 3 NOLD cases had normal GBs and 2 were small.

Conclusions: All NOLD cases lacked definitive features of an obstructive pattern of injury at an early age (5-10 wks) and had GBs, but end-stage histologic findings are otherwise similar and clinical features overlap with BA. All cases showed central venous sclerosis, minimal periportal copper deposits, and increased lymphatics, features previously under-recognized in BA. The similarity of these findings suggest that at least some cases of NOLD may represent a delayed presentation or evolution of BA, and presence of GB may be associated with these outlier cases.

1432 Clinicopathologic Features of CMV Hepatitis in Liver Transplant Patients

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Background: CMV is the most commonly encountered opportunistic viral infection in liver transplant patients, typically seen 4-8 weeks after transplantation. The usual histologic features have been described and include interface and lobular hepatitis, lobular neutrophilic microabscesses, microgranulomas, and characteristic intranuclear and/or cytoplasmic inclusions. It has been noted that with current prophylaxis regimens, inclusions may be less prominent; therefore, IHC stains have become more important in identifying CMV hepatitis. We aim to further characterize the histologic, IHC, and clinical features of CMV in transplant patients.

Design: 55 needle core biopsies from 50 patients (1990-present) were reviewed. Biopsies were scored for rejection activity index, interface and lobular activity, number and location of CMV inclusions, neutrophilis, microgranulomas, and granulomas. We examined IHC, when performed, and the number of positive cells was compared to the H&E.

Results: The diagnosis of CMV hepatitis was made within 12 weeks of transplant in 60%, 13-24 weeks in 27%, and >24 weeks in 13%. The number of CMV inclusions identified by H&E staining varied widely ranging from 0-185. There were no inclusions in 9% and only one inclusion in 9%. Inclusions were seen on only 1 of 3 H&E levels in 5%. On average, there were 8 times more CMV inclusions found on IHC than on H&E. In the 5 cases where inclusions were not seen by H&E, the histologic diagnosis of acute cellular rejection could be made in 2 cases, biliary obstruction in 1, chronic rejection in 1, and non-specific hepatitis in 1. IHC was ordered in these cases due to serum CMV PCR positivity in 3, neutrophilic infiltrates in 1, and microgranulomas in 1. Inclusions were seen in hepatocytes in 96%, endothelial cells in 44%, and biliary cells in 18%. In cases with endothelial inclusions, 67% had endothelitis. In contrast, in cases without endothelial inclusions 23% had endothelitis. In the cases with biliary inclusions, 50% had biliary obstructive features. In contrast, in cases without biliary inclusions 16% had biliary obstructive features. Well formed neutrophilic microabscesses were present in 49%, irregular collections of neutrophils in 22%, microgranulomas in 49%, and granulomas in 9%.

Conclusions: Because a significant number of cases (18%) had no or a single inclusion, and because neutrophilic microabscesses are frequently not present, one must have a low threshold for performing IHC and suggesting serum PCR.

1433 Histologic Findings in Post-Reperfusion and Follow-Up Biopsies in Liver Transplant Patients: Is There Any Correlation?

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Background: Currently, there is no consensus to perform time zero biopsy in liver transplant (LT) setting. In this study, we examined the donor liver biopsies at the time of transplant, and evaluated progress of pathologic findings in subsequent biopsies.

Design: Between Jan-May 2007, we selected 40 consecutive liver transplant patients. All patients underwent protocol liver biopsies at the end of the LT procedure. The post-reperfusion & follow-up biopsies were reviewed by one expert pathologist.

Results: Only patients with at least one follow-up biopsy (20 males and 7 females) were included in the study & a total of 260 pathology slides (H&E, iron & trichrome stains) were reviewed. Mean age was 52.4 years (SD±9.5) in the recipient group and 44.2 years (SD±17.5) in the donor group. Mean cold & total ischemic time were 47 and 443.5 minutes (SD±12.38; SD±104.10), respectively. Mean follow-up time was 197.2 days (SD±35.7). Mean time between post-reperfusion biopsy & follow-up biopsy was 55.4 days (SD±51.1) for the first, 110 days (SD±58.1) for the second, & 93 days (SD±61.7) for the third follow-up biopsies. Histologic findings in post-reperfusion biopsies consisted of: Mild to moderate preservation injury (18 cases), micro- & macrovesicular steatosis (6 & 4 cases, respectively), mild periportal chronic inflammation and fibrosis (3 cases), & pericellular fibrosis (3 cases). In follow-up biopsies, major histologic findings included acute cellular rejection (5 cases) and recurrent hepatitis C (5 cases). Of those histologic findings in post-reperfusion biopsies, only the following pathology were present in subsequent biopsies: Mild preservation injury (2 cases), minimal chronic periportal inflammation & fibrosis (2 cases), and mild residual micro- and macrovesicular steatosis (1 case each).

Conclusions: This preliminary report indicates that in most instances, the histologic findings in post-reperfusion liver biopsy are subtle and do not persist in follow-up biopsies. This may question the value of time zero biopsy. However, there are some instances whereby the pathologic process cannot be assessed in follow-up biopsies without having a baseline, especially when it comes to degree of steatosis and fibrosis. Given the low morbidity of such biopsies and continued expansion of donor criteria, post-reperfusion biopsy is appropriate to better understand the long term impact of baseline pathologic findings.

1434 Pancreatic Lymphoepithelial Cysts: A Morphologic and Immunohistochemical Study

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Background: Pancreatic lymphoepithelial cysts (LECs) are rare benign cysts that are typically lined by mature, keratinizing squamous cells with surrounding lymphoid tissue. LECs cannot be reliably differentiated from neoplastic mucinous cysts preoperatively and diagnostic studies can be misleading. If squamous cells and keratin are lacking, or if atypical glandular cells are present, FNA may not be diagnostic or may suggest a mucinous neoplasm. CEA and CA19-9 are often elevated in the cyst fluid, which favor a mucinous cyst. In order to clarify potential pitfalls in the preoperative diagnosis of LEC, a morphologic and immunohistochemical study was performed on resected cysts.

Design: We studied 7 resected LECs. 4 were from men and the mean age was 58 yrs. Stains for CEA, CA19-9, CK7, p63, PASD and apomucins (MUC1, MUC2, MUC4, MUC5AC and MUC6) were performed. The pathology data was correlated with clinical information and prior FNA results.

Results: The LECs were located in the body (2), head (2), tail (1) and peripancreatic region (2); 4 were multilocular. The mean cyst size was 4.8 cm (range 3-10 cm). On FNA, 5 had squamous cells or keratin debris; all 7 had glandular cells and 4 of these had cellular atypia. Serum CEA levels were normal (n=4); serum CA19-9 was mildly

elevated in 1 patient (n=3). Cyst fluid CEA was 493 ng/mL (normal <5ng/ml) in the 1 cyst that was tested. Morphologically, all cysts were lined by mature, stratified squamous cells and produced keratin. In 6, there were focal to diffuse areas where the lining resembled transitional epithelium. The presence of surface mucinous cells has not been well-reported in these cysts. On the H&E and PASD stains, 5 (71%) cysts contained mucinous cells (scattered in 4, rare in 1); 2 showed none. Predominantly surface staining of the squamous cells was seen with CK7, MUC1 and MUC4 in all cysts (100%), an expected result in transitional epithelium, and with CEA and CA19-9 in 6 cysts (86%) (the mucinous cells were negative for MUC1 but positive for the others and MUC5AC). All cysts were positive for p63 and negative for MUC2 and MUC6.

Conclusions: LECs have morphologic and immunophenotypic features that could lead to a misdiagnosis preoperatively. Though these cysts are typically lined by keratinizing squamous cells, we showed that 71% of our cysts also had at least focal mucin-producing cells. Interestingly, the squamous cells in 86% of the cases were positive for CEA and CA19-9, which explains the positive cyst fluid analyses that have been reported in these cysts and creates a potential diagnostic pitfall.

1435 The Grade, Extent, and Duct Involvement of PanIN Associated with Pancreatic Ductal Adenocarcinoma and IPMN Differ from Those Associated with Non-Ductal Pancreatic Tumors and Non-Turmorous Lesions

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Background: Pancreatic Intraepithelial Neoplasia (PanIN) is considered to be a precursor to pancreatic ductal adenocarcinoma (PDA). PanINs have also been found in association with other non-PDA tumors and non-tumorous lesions, but the characteristics of PanINs associated with those lesions are not well characterized.

Design: Patients who underwent partial or total pancreatectomy from1994 to 2006 were identified from the Pathology database in the Mount Sinai hospital. A total of 156 cases with available clinical history and histological slides were selected. Among them were 74 PDAs, 13 intraductal papillary-mucinous neoplasms (IPMNs), 49 non-ductal tumors (NDT) (19 islet cell tumors, 12 serous cystadenomas, 2 mucinous cystadenomas, 2 solid pseudopapillary tumors, 1 granular cell tumor, 8 ampullary tumors, and 3 metastatic carcinomas), and 20 non-tumorous lesions (NTL)(12 chronic pancreatitis, 7 incidental pancreatectomy, and 1 severe acute cellular rejection). The differences of the grade, extent, and duct involvement among PanINs associated with different lesions were analyzed by Chi square test.

Results: The median age of patients with PDA, IPMN, NDT, and NTL was 67 years (range 27-88), 69 years (range 34-81), 58 years (range 23-80), and 53 years (range 29-81), respectively. PanINs were found in 93%, 92%, 59% and 50% pancreata with PDAs, IPMNs, NDTs, and NTL, respectively. High-grade PanINs (grade III) were more commonly associated with PDAs (51%) and IPMNs (31%) than those with NDTs (2%) and NTLs (5%) ($p< 0.01$). There was a higher percentage of PanINs involving more than 33% ducts in PDAs (44%) and IPMNs (38%) than those in NDTs (12%) and NTL(10%) ($p<0.01$). PDAs and IPMNs-associated PanINs were more commonly involved in the main ducts (54%) than NDTs and NTLs associated (22% and 20%, $p<0.05$). No statistical differences were observed between PanINs associated with PDAs and IPMNs and between those with NDTs and NTLs ($p>0.05$).

Conclusions: Compared to PanINs associated with PDAs and IPMNs, those associated with NDTs and NTLs usually had a lower prevalance, lower grade, less extensive involvment of ducts, and a propensity to involve branches. Results suggest that high-grade PanINs is more closely related to PDAs or IPMNs, whereas low-grade PanINs might be attributable to a compound effect, such as aging.

1436 Does Knowledge of Immune Functioning Aid in the Interpretation of Liver Allograft Biopsies in Patients Status Post Liver Transplantation for Viral Hepatitis C?

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Background: Liver allograft biopsy is the current gold standard for distinguishing between recurrent viral hepatitis C and acute cellular rejection. Occasional biopsies, however, reveal mixed or subtle microscopic findings and the histologic interpretation of these cases can be challenging. Because the therapies differ by diagnosis, an incorrect diagnosis can lead to disease exacerbation and graft injury. Correlation of biopsy findings with ancillary laboratory tests may aid in the biopsy interpretation. Immunokinetic assays, based on in vitro stimulation of patient T lymphocytes with quantification of ATP produced may be a better reflection of a patient's level of immune function than immunosuppressant drug levels. We sought to correlate the histologic findings in liver allograft biopsies with immunokinetic assay results.

Design: We identified 17 patients who were status post liver transplantation for hepatitis C who underwent liver biopsy for elevated liver function tests, had evidence of inflammation on biopsy, and had concomitant immunokinetic assay testing. The chronic hepatitis necroinflammatory grade (incorporating lymphocytic piecemeal necrosis and lobular inflammation / necrosis) and rejection activity index (sum of portal inflammation, bile duct injury, and endothelialitis) were compared to the immunokinetic assay result using standard parametric and non-parametric statistical evaluation.

Results: 19 liver biopsies were identified from patients a median of 12 months post liver transplant (range 0.2-200 months). Mean immunokinetic values (ng/ml ATP) for Grade 0, 1, 2, 3 chronic hepatitis were 430.0, 240.6, 171.8 and 53.0 respectively ($p=0.02$). The immunokinetic assay results inversely correlated with the total necroinflammatory grade of chronic hepatitis ($r=-0.68$ $p=0.002$). There was no relationship between the level of immune response and the total RAI score ($p=0.96$). Results were the same for both parametric and non-parametric statistical tests.

Conclusions: In patients who are status post liver transplantation for hepatitis C, incorporation of immunokinetic assay results may aid in the interpretation of inflammation in liver allograft biopsies with subtle or mixed histologic findings.

1437 Nonalcoholic Steatohepatitis Associated with Obesity: Histological Evolution after Bariatric Surgery

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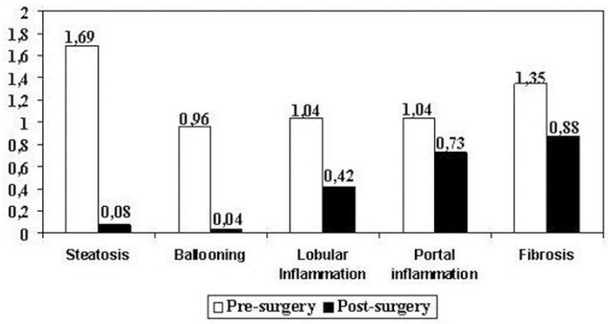
Background: Steatohepatitis is a morphological pattern of liver injury that may be seen in obese patients. The purpose of this study was to determine the morphological changes and the prognostic factors of nonalcoholic steatohepatitis (NASH) in obese patients after bariatric surgery (BS).

Design: 17 patients (13 females), 11 with metabolic syndrome, were submitted to BS. Two hepatic biopsies were performed: first biopsy at the surgical treatment and the second after a median of 13 months (confidence interval: 12-18m.) post-surgery. Clinical, biochemical and histological variables were considered in both biopsies

Results: Based on well-recognized histopathological features: fatty liver alone and NASH, which includes hepatocellular steatosis and ballooning, mixed acute and chronic lobular inflammation, and perisinusoidal or portal fibrosis according to Elisabeth M. Brunt index 2005, our results were: First biopsy: fatty liver alone (n=1, 6%), nonalcoholic steatohepatitis (n=16, 94%). Second biopsy: steatosis grading (1.6±0.8 vs, 0.06±0.2; $p<0.001$), ballooning (0.5±0.7 vs 0.06±0.2; $p=0.007$), lobular inflammation (0.9±0.2 vs 0.2±0.4; $p< 0.003$), portal inflammation (1.1±0.4 vs 0.7±0.3; $p=0.002$) and fibrosis (1.3±0.8 vs 1.0±0.9 $p=0.009$). 5 patients persisted with significant fibrosis (F>1) after BS. The prognostic factors associated with fibrosis persistence were: elevated transaminases (AST) level preBS ($p=0.034$), steatosis grading ($p=0.002$) and fibrosis staging.($p=0.001$), in the first hepatic biopsy.



Conclusions: 1. Bariatric surgery for obese patients improves histological lesions, mainly the steatosis grading. 2. Elevated transaminases (AST) level, high steatosis grading and fibrosis staging (F>2) in the first biopsy are prognostic factors related to fibrosis persistence.

1438 Divergent Activation of the DNA Damage Response in Ductal and Endocrine Neoplastic Progression in the Pancreas

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Background: Neoplastic progression in the pancreas is associated with the accumulation of genetic and epigenetic changes that result in DNA damage and genomic instability. While lineage differentiation from a common stem cell is operative in pancreatic development, the molecular pathways involved in endocrine and ductal derived neoplasms differ. H2AX is a histone protein which becomes phosphorylated in response to DNA double-strand breaks and is a marker of an activated DNA damage response. We hypothesized that staining for phosphorlyated H2AX (γ-H2AX) would increase with neoplastic progression in the pancreas.

Design: Cases of normal pancreas (n=9), chronic pancreatitis (n=9), intraductal papillary mucinous neoplasia (IPMN) (n=10) and pancreatic endocrine tumor (n=10) were selected from the Hospital of the University of PA Pathology archives and stained with a polyclonal antibody against phosphorylated H2AX (γ-H2AX) using standard immunohistochemical techniques. For quantitative analysis, fifty high power fields, or all available lesional tissue if less, were assessed for the number of positive-staining nuclei and the total number of cells. For normal controls, ducts from unaffected pancreas in pancreatic endocrine tumor and islet cells from microcystic adenoma were similarly assessed. The percentage of γ-H2AX positive cells (%γ-H2AX) was calculated, the mean and median for each lesion type determined, and Student's t-test applied.

Results: There was an increasing %γ-H2AX in normal ducts, ducts in chronic pancreatitis, and in IPMN (mean[SE]/median: 0.05[0.02]/0.03, 0.48[0.29]/0.15, 0.28[0.06]/0.25). The %γ-H2AX in IPMN was significantly different than normal ($p=.002$). In contrast, while the %γ-H2AX increased in islet cells in chronic pancreatitis (0.88[0.72]/0.175), the endocrine tumors (0.19[0.09]/0.06) did not differ compared to normal (0.08[0.02]/0.07).

Conclusions: Staining for γ-H2AX suggests that activation of a DNA damage response occurs in chronic pancreatitis in both the ductal and islet cells. DNA double-strand breaks may be important genetic events in the development of the ductal derived IPMN tumors, but not in pancreatic endocrine tumors, further showing their divergent molecular pathways.

1439 Immunohistochemical and FISH Analysis of EGFR and HER-2/neu Status in Adenocarcinomas of the Biliary Tree and Gallbladder

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Background: Adenocarcinomas of the biliary tract and gallbladder are aggressive tumors with a poor prognosis and 5-year survival of <5%. Standard chemotherapy often offers minimal benefit. Since EGFR and HER-2/neu antagonists have been successfully used in adenocarcinomas from other sites, their use in cholangiocarcinoma can be potentially

beneficial. This study examines the EGFR and HER-2/neu expression, and EGFR gene copy number in biliary tract adenocarcinomas.

Design: 37 formalin-fixed paraffin-embedded cases of adenocarcinomas (25 intra-hepatic, 10 extra-hepatic, 2 gallbladder) were stained with monoclonal antibody against EGFR (Clone 2-18C9), using the EGFR pharmDx kit (DAKO, Carpinteria, CA) and with monoclonal antibody (CB11) against HER-2/neu (Ventana, Tucson, AZ). Cell membrane staining was recorded as weak (1+), moderate (2+) and strong (3+). Cases with 2+ or 3+ staining in >10% of cells were considered positive. For EGFR fluorescence in situ hybridization (FISH) analysis, Spectrum Orange-labeled EGFR probe (Vysis, Downers Grove, IL) and Spectrum Green-labeled probe against the centromeric region of chromosome 7 (CEP 7) was hybridized to 5µm sections and counterstained with 2-6-diamidino-2-phenylindole (DAPI).

Results: Moderate or strong EGFR staining (2+ or 3+) in greater than 10% of tumor cells was seen in 22 (59%) cases. HER-2/neu expression (2+) was seen in 1 (3%) case; no tumor showed 3+ staining. FISH analysis in 11 cases showed gain in EGFR gene copy number in 3 (27%) tumors. Of the latter, 2 showed gain in chromosome 7 indicating balanced polysomy, and 1 showed gene amplification. All 3 cases showed EGFR overexpression by immunohistochemistry. Of the 8 cases with normal EGFR gene copy number, EGFR overexpression by immunohistochemistry was seen in 4 (50%) cases.

Conclusions: EGFR is moderately or strongly overexpressed on the cell membrane in the majority of adenocarcinomas of the biliary tree. Gain of EGFR gene copy number in the form of balanced polysomy or amplification is present in a minority of cases. EGFR overexpression by immunohistochemistry was seen in all cases with extra gene copy numbers, but also in half of the cases with normal gene copy numbers. EGFR expression in the majority of these tumors suggests that they may respond to treatment with EGFR antagonists. HER2/neu expression is uncommon in these tumors.

1440 *KRAS2* Gene Mutations in Acinar-Ductal Metaplasia in the Pancreas: Pancreatic Neoplasia May Start Prior to Pancreatic Intraepithelial Neoplasia Lesions

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Background: Pancreatic intraepithelial neoplasm (PanIN) is a precursor to invasive ductal adenocarcinoma of the pancreas. Mutations in *KRAS2* proto-oncogene are thought to be early events in the development of PanIN lesions. Observations made in genetically engineered mouse models suggest that the acinar/centroacinar compartment can give rise to ductal neoplasia. In order to integrate findings in mice and men, we examined human acinar cells, acinar-ductal metaplasia lesions and PanINs for *KRAS2* gene mutations to determine if *KRAS2* gene mutations occur before the development of PanINs in human pancreata

Design: Surgically resected pancreata were screened for PanIN lesions associated with acinar to ductal metaplasia. PanIN lesions, acinar-ductal metaplasia, and acinar cells from the same lobule, as well as stromal cells were microdissected using laser capture microdissection. Genomic DNA from the microdissected tissues was subjected to nested PCR amplification of *KRAS2* gene followed by PCR DNA sequencing.

Results: Seventeen sets of lesions from 16 surgically resected pancreata were analyzed. *KRAS2* gene mutations at codon 12 were present in 8 of 17 (47%) PanIN lesions. In 4 of these 8 cases (50%) with *KRAS2* mutations in PanINs, the same *KRAS2* mutation was present in the acinar-ductal metaplasia lesion associated with the PanIN. The remaining acinar-ductal metaplasia lesions, all of the adjacent acinar cells and all control stromal cell samples were *KRAS2* wild type.

Conclusions: Human pancreatic neoplasia may originate from foci of acinar-ductal metaplasia prior to pancreatic intraepithelial neoplasia lesions.

1441 Glypican-3 Shows Intermediate Sensitivity between Hepatocyte and Alpha-Fetoprotein: A Tissue Microarray Study of 809 Carcinomas

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Background: The distinction between hepatocellular carcinoma (HCC) and metastatic carcinoma can be difficult. Glypican-3 (Gly3) is a member of the heparin sulfate proteoglycan family and is overexpressed in many HCCs but not in benign adult liver. We compared Gly3 to Hepatocyte (Hep) and Alpha-Fetoprotein (AFP) to determine the most useful stain for this distinction.

Design: Tissue microarrays from 732 carcinomas from lung (142), thyroid (31), prostate (49), pancreaticobiliary (104), pancreatic neuroendocrine (15), breast (19), endometrium (267), colon (105); 77 HCC; and 10 hepatocellular adenomas (HA) with cores ranging from 0.6 to 2.0 mm were stained with antibodies against Gly3, Hep, and AFP. Controls stained appropriately and stains were interpreted by 2 pathologists. Greater than or equal to 5% staining was considered positive. The sensitivity and specificity for each stain were determined for the ability to distinguish hepatocellular from other sites of origin.

Results: The table compares staining in HCC, other carcinomas and HA. Gly3 was positive in 46/74 (62.2%) HCC, 6/100 (6%) pancreaticobiliary carcinomas, 2/15 (13.3%) pancreatic neuroendocrine tumors, and 4/94 (4.3%) colon carcinomas and negative in all other tumors. Hep was positive in 62/75 (82.7%) HCC, 2/137 (1.5%) lung carcinomas, 10/94 (10.6%) pancreaticobiliary carcinomas, 2/105 (1.9%) colon cancers, 9/10 (90%) HA and negative in the other tumors. AFP was positive in 15/74 (20.3%) HCC and 1/142 (0.7%) lung carcinomas and negative in all other tumors. The sensitivity and specificity for HCC vs. other carcinomas was: for Gly3, 62% and 98%; for Hep, 83% and 98%; and for AFP, 20% and 99.9%. Gly3 and AFP were negative in all HA while Hep stained 9 of 10 (90%) HA.

Conclusions: All three stains are very specific for hepatocellular differentiation in malignant tumors, but Hep shows excellent sensitivity while Gly3 is intermediate and AFP is poor. Although Hep appears much more useful for the distinction between malignant tumors in the liver, Gly3 offers the advantage of being useful to differentiate malignant from benign hepatocellular lesions. Therefore, Gly3 may be a useful addition to panels to aid in the distinction of tumors in the liver.

Comparison of Immunohistochemical Staining

Stain	HCC (n=77)	Other Carcinoma (n=732)	HA (n=10)
Gly3	46/74 (62.2%)	12/688 (1.7%)	0/10 (0%)
Hep	62/75 (82.7%)	14/706 (2.0%)	9/10 (90%)
AFP	15/74 (20.3%)	1/707 (0.1%)	0/10 (0%)

1442 Glypican-3 Is More Helpful Than CD34 but Not as Useful as Reticulin To Discriminate Hepatocellular Carcinoma from Adenoma

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Background: Hepatocellular carcinoma (HCC) can be difficult to distinguish from hepatocellular adenoma (HA) or benign liver. Reticulin staining is useful to confirm widened cell plates in HCC. Immunohistochemical stains, such as CD34, to highlight sinusoidal endothelial cells in HCC have also been suggested. Glypican-3 (Gly3) is an oncofetal protein overexpressed in many HCCs but not in benign adult liver or HA. We compared Gly3 to reticulin and CD34 to determine the most useful stain for this distinction.

Design: Forty-eight cases of HCC and 9 HA were retrieved from archival files. Tissue cores of lesions (57 cases) and uninvolved adjacent liver (39 cases) from formalin-fixed, paraffin embedded donor blocks (2 cores per block) were arrayed to create a tissue microarray of cores measuring 2.0 mm each. Sections were stained with Gly3, reticulin and CD34. Stains were interpreted by 2 pathologists as negative (benign pattern) or positive (malignant pattern) as follows: Glyp3, <5%, benign and ≥5% malignant; reticulin, ≤3 cell wide plates benign and >3 malignant; CD34, periportal benign and more diffuse sinusoidal malignant. Controls stained appropriately and sensitivity, specificity, positive and negative predictive values were determined.

Results: The table shows positive cases (malignant pattern) for each group. HA and uninvolved liver showed a benign pattern with reticulin and Gly3, while 8/9 HA and 5/39 uninvolved liver showed a malignant pattern with CD34. For the distinction of HCC from HA sensitivity, specificity, positive and negative predictive values were: for reticulin, 100% each; for Glyp3, 65%, 100%, 100%, 64%; and for CD34, 96%, 11%, 85%, 33%, respectively.

Number of Cases Showing Malignant Staining Pattern

Stain	HCC (n=48)	HA (n=9)	Adjacent Liver (n=39)
Glypican-3	31/48 (65%)	0/9 (0%)	0/39 (0%)
Reticulin	48/48 (100%)	0/9 (0%)	0/39 (0%)
CD34	46/48 (96%)	8/9 (89%)	5/39 (12%)

Conclusions: Reticulin is the most useful stain to distinguish HCC from HA. Gly3 is equally as good when positive, but negative staining does not exclude HCC. CD34 is not useful in this distinction, although it is negative in most nonlesional liver and may help confirm the biopsy is from the lesion. Although reticulin is more useful than Gly3, Gly3 is more specific for hepatocellular differentiation, since widened cell plates may occur in neoplasms of non-hepatic origin. Therefore, Gly3 may be useful as part of a panel together with reticulin and H&E in difficult cases.

1443 Pancreatic Intraepithelial Neoplasia Is Common at the Periphery of and Can Be Present within Serous Cystadenomas

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Background: Incidental foci of pancreatic intraepithelial neoplasia (PanIn) are common in pancreas resections, and when found away from the lesion of interest, pose no diagnostic dilemmas. PanIn has been anecdotally noted at the periphery of and within serous cystadenomas (SC) at our institution, posing diagnostic difficulty at frozen section in one patient. The incidence and significance of PanIn found specifically at the periphery of and within non-mucinous tumors of the pancreas has not been studied previously. We report the frequency of PanIn associated with SC at our institution.

Design: A search of the pathology database revealed 16 SC resections with available slides between 1984 and 2007. Sections were scored by two pathologists simultaneously for PanIn grade and frequency on a 1-3+ scale. PanIn foci were categorized as being within, at the periphery of, or distant from the SC. PanIn was deemed distant if intervening pancreatic parenchyma was present between the focus of PanIn and the SC.

Results: 9/16 (56%) of SC contained PanIn at the tumor periphery. PanIn grade was I in 8, II in 2 and III in 1 case. 1/16 7% of SC contained PanIn 1 both at the periphery and within the SC. In that case, several individual glands were lined by both serous and mucinous cells, and a closely shaved frozen surgical margin revealed several dilated mucinous glands, causing initial confusion with intraductal papillary mucinous tumor (IPMN). In another case, permanent sections revealed a single dilated mucinous gland with dysplasia at the periphery of the SC, that was diagnosed as an incidental IPMN. 12/16 (75%) of SC contained PanIn distant from the lesion. Size of the SC did not correlate with the frequency, location or grade of PanIn found.

Conclusions: PanIn of varying grades is common at the periphery of SC, may be present at surgical resection margins on frozen section, and may be histologically indistinguishable from a side branch IPMN. Rarely, PanIn may be present within SC tumors, where single glands with both serous and mucinous lining may be found. Whether the PanIn foci represent a reaction to mass effect, or precede tumor development is unknown. The relationship between PanIn and IPMN like lesions in the setting of SC merits further study. Pathologists should be aware of this association when evaluating margins and permanent sections of SC.

Number of PanIn foci per case of Serous Cystadenoma

# of Foci	Periphery	Within	Distant
1-5	5	1	5
6-10	3	0	4
>10	1	0	3

1444 Variants of Nonalcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH) with Rapidly Progressive Course

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Background: NAFLD and NASH typically manifest as slowly progressive chronic liver disease. We now report four cases with a rapidly aggressive clinical course, and document the unique clinical and histologic features.

Design: Within a 12 month period, 4 patients with rapidly progressive fatty liver disease manifesting as fulminant or sub-fulminant liver failure were identified. Medical records and liver biopsies were reviewed and findings including steatohepatitis (per Kleiner et al), degree/pattern of fibrosis, cholestasis, Mallory bodies, and ductular reaction were identified and correlated with clinical course and laboratory findings. Other causes for liver failure, including alcohol use, were excluded.

Results: Patients were women (3 of Hispanic ethnicity) between the ages of 25 and 41, all with history of morbid obesity (BMI range from 39.7 to 59.4 kg/m^2), followed by rapid weight loss (varying from 50-200 lbs. in 6 mon to 3 yr) after bariatric surgery or starvation-like dieting. All presented with fulminant or subfulminant liver failure, including severe hepatic synthetic dysfunction (marked hyperbilirubinemia, coagulopathy and hypoalbuminemia) and encephalopathy. On biopsy, 1 patient had reversible findings of grade 3 macrovesicular steatosis, no definitive fibrosis, minimal lobular inflammation, and only rare ballooned hepatocyte. The other 3 had advanced disease, resulting in death or liver transplantation, with extensive centrizonal sinusoidal fibrosis, bridging central/central and central/portal fibrosis (stage 3-4), panacinar sinusoidal fibrosis, and cholestasis with centrizonal and periportal ductular reaction. Ballooned hepatocytes, Mallory bodies, and lobular neutrophils were abundant. Fat was present in varying degrees from grade 1-3. One of these 3 patients had a "long-limb" Roux-en-Y gastric bypass associated with profound weight loss 5 months prior to liver failure. Biopsy at that time showed mild steatohepatitis and minimal centrizonal fibrosis, confirming rapid progression of the lesion from Stage 1a to 3.

Conclusions: NAFLD and NASH can present as rapidly progressive liver failure. The histologic findings in the advanced cases are more typical of alcoholic injury (abundant Mallory bodies, neutrophils, cholestasis, pattern of fibrosis) than the usual NAFLD. This study suggests rapid weight loss and female gender as possible risk factors for these variants. The type of bariatric surgery and Hispanic ethnicity may also be contributing factors.

1445 Tumor Infiltrating Neutrophils in Pancreatic Neoplasia: Biologic Implications

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Background: The importance of the interaction between the tumor cells and inflammatory cells in cancer initiation and progression is becoming increasingly recognized. It has, however, not been systematically investigated in pancreatic neoplasia.

Design: The presence of neutrophils concentrating within and/or adjacent to neoplastic cells (tumor infiltrating neutrophils; TINs) was investigated in various pancreatic tumor types resected. Foci of inflammation with prominent stromal neutrophilia were disregarded. >10 TINs/100 epithelial cells were arbitrarily classified as significant.

Results: In 363 invasive carcinomas analyzed, 15 showed significant TINs; 8 of these had micropapillary pattern and remaining 7 were undifferentiated ca.Among other pancreatic tumors, significant number of TINs was seen almost exclusively in the ***in-situ papillary carcinomas with pancreatobiliary pattern***. Of the 19 MCNs with in-situ papillary component, 11 (60%) showed TINs (mean, 25 PMNs/100 epithelial cells, ranging from 14 to 63). Furthermore, among IPMNs, significant TINs were identified in 4/16 of pancreatobiliary type papillae, but was very uncommon and less prominent in other types (1/11 of oncocytic and 1/23 intestinal types each had TINs of <15, respectively, and none of 10 of gastric-type papilla had any. Low-grade and non-papillary components of these tumors did not show any TINs. No striking TINs was noted in 40 endocrine tumors, 18 serous adenomas, 9 acinar cell ca., or 8 solid-pseudopapillary tumors. 3/14 colloid ca had mild neutrophilia.

Conclusions: Significant amount of tumor infiltrating neutrophils is uncommon in ductal adenocarcinoma of the pancreas, and when it occurs it is in either micropapillary or undifferentiated patterns. Among pre-invasive neoplasia, TINs appear to show predilection for papillary in-situ carcinomas of MCNs, or less commonly, pancreatobiliary type IPMNs (both being MUC1 expressing papillae). This may have biologic implications, especially considering the recent literature on the close interaction of MUCs and inflammatory cells in carcinogenesis, and the well known differential expression of MUCs in pancreatic neoplasia. The predilection of TINs for MCNs is also interesting, considering the strong analogy with the so-called "endocervical" subset of ovarian MCNs which also show TINs used as one of the diagnostic criteria.

1446 Pan-Hypopituitarism: An Under-Recognized Cause of Neonatal Giant Cell Hepatitis

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Background: Neonatal giant cell hepatitis (NGCH) is an important diagnostic consideration when evaluating liver biopsies performed in infants who present with jaundice. NGCH is a pattern of liver injury that can result from multiple etiologies, though most cases are idiopathic. In this study we report an under-recognized cause of NGCH: pan-hypopituitarism.

Design: All liver biopsies (1984 to 2007) with a histological diagnosis of NGCH from a single large referral center were reviewed. Cases diagnosed histologically as biliary atresia, paucity of intrahepatic bile ducts, TPN induced cholestasis, and alpha-1-antitrypsin deficiency were excluded. Liver biopsies were retrospectively examined, while blinded to clinical information, for the following: cholestasis, giant cell change, extra-medullary hematopoiesis, inflammation, and fibrosis. Follow-up clinical and laboratory findings were subsequently reviewed.

Results: 32 cases of NGCH were identified (66% male). The average age at liver biopsy was 2 months. The biopsies averaged 12 mm in size with 9.5 portal tracts. The biopsies typically showed mild to moderate cholestasis in a predominately canalicular pattern (67% of cases) with no evidence for ductopenia (100%). Focal mild bile ductular proliferation was seen in 30% of cases while moderate ductular proliferation was present in a single case. The proportion of hepatocytes involved by giant cell change ranged from 10-60%, average 30%. Extra-medullary hematopoiesis was typically prominent (95% of cases) while portal and lobular inflammation was mild to absent in 31/32 cases. Portal fibrosis was usually absent but was mild in 2 cases and equivocal in another 2 cases. Pericellular fibrosis was also typically absent but was focal in 3 cases and severe in 1 case. Only a single case showed minimal macrovesicular steatosis. Subsequent clinical follow-up and additional testing showed the following etiologies: Idiopathic (N=19), pan-hypopituitarism (5), biliary atresia (2), Alagille syndrome (2), progressive familial intrahepatic cholestasis (1), severe combined immunodeficiency (1), urine positive for CMV (1), blood positive for Echovirus 11 (1). The biopsy findings did not distinguish between the different etiologies.

Conclusions: Pan-hypopituitarism is an important but under-recognized cause of neonatal giant cell hepatitis. The majority of cases of neonatal giant cell hepatitis remain idiopathic and histological features do not readily distinguish amongst various etiologies.

1447 Staining for HMGA2 in Serous and Mucinous Cystic Pancreatic Lesions

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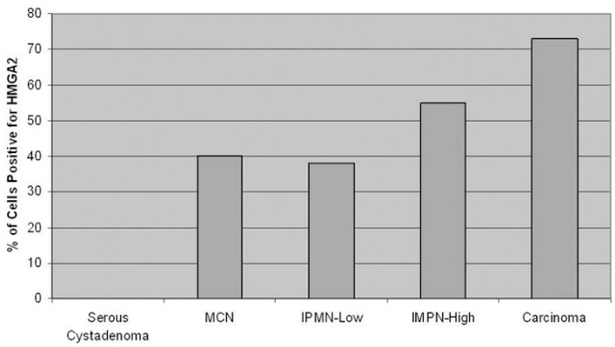
Background: HMGA2 is a member of the DNA-binding protein family HMGI which plays a role in regulating transcription in early differentiation and proliferation during development. While there is no significant expression in normal adult tissues, HMGA2 has an increased expression in oral, breast, and pancreatic carcinomas. The related protein HMGI(Y) has additionally shown increased expression in intraductal papillary mucinous tumors (IPMN) of the pancreas. As the number of asymptomatic pancreatic lesions detected with advances in imaging has increased, there has been an increased need to evaluate cystic pancreatic masses. Our hypothesis is that HMGA2 will show increased staining in lesions with an increased malignant potential.

Design: We performed immunohistochemical staining for HMGA2 on cases of IPMN (18 cases), mucinous cystic neoplasms (MCN, 5 cases), serous cystadenomas (4 cases), as well as chronic pancreatitis (4 cases) and pancreatic cancer arising from IPMNs (12 cases). The percentage of positively staining cells was assessed. Cases in which there was less than 10% staining were interpreted as being negative.

Results: All serous cystadenomas were negative for HMGA2 staining. 4 of 5 MCNs showed positive staining, with an average of 40% of cells staining. IPMNs were positive in 15 of 18 cases. Within the positive cases the percentage of cells staining increased progressively with increasing atypia. Low-grade lesions showed 38% positivity. High-grade lesions showed 55% positivity. All cases of carcinoma showed HMGA2 staining, with 73% of cells staining positively. Chronic pancreatitis showed positive staining in 3 of 4 cases, with preferential staining of small proliferative ductules. The percentage of cells staining in positive cases is given in figure 1.

Figure 1



Conclusions: HMGA2 is useful in distinguishing serous and mucinous neoplasms, being uniformly negative in the former. All mucinous lesions showed positive staining in a majority of cases, whether benign or malignant. Among IPMN tumors, we found increased staining for HMGA2 with increasing malignant potential.

1448 *ABCB4* Mutation and Intrahepatic Cholestasis: Cholesterol Crystals in Bile Canaliculi

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Background: Multidrug resistance protein 3 (MDR3), encoded by *ABCB4*, moves phosphatidylcholine (PC) from inner to outer layer of the bile canaliculus (BC) membrane. Thence PC enters bile. Cholesterol (CH) dissolves poorly in PC-deficient bile, as in mice lacking the MDR3 orthologue Mdr2 ("*Abcb4* -/- mice"). Hepatic bile from such mice fed a semi-synthetic diet (SSD) plus 0.5% cholate (SSDC) contains

CH crystals. MDR3 deficiency is associated with CH gallstones, CH sludging in bile ducts, small-duct sclerosing cholangitis, and intrahepatic cholestasis (IC), of infancy or with hormonal shifts. Approaches to morphologic diagnosis of MDR3 deficiency are not defined.
Design: Our 3 non-related study subjects (SS) were a 31-year-old with IC of pregnancy and 2 infants with IC. We assessed liver biopsy specimens by light microscopy (LM), with immunostaining (IS) for MDR3 and a homologue, multidrug resistance-associated protein 2 (MRP2), and by transmission electron microscopy (TEM). We sequenced *ABCB4* exons and exon-intron junctions. Liver from infants with Alagille syndrome, biliary atresia, and neonatal sclerosing cholangitis was compared (n = 2 each; "comparison subjects" [CS]). We performed LM and TEM of liver of wild-type mice fed standard chow, *Abcb4* -/- mice fed SSD, and *Abcb4* -/- mice fed SSDC (n = 2 each).
Results: On LM, all humans and *Abcb4* -/- mice had cholestasis / cholangiopathy. The woman SS proved heterozygous, and each infant SS homozygous, for *ABCB4* mutations predicted to disrupt MDR3 synthesis (woman, c.551T>C / p.Ile184Thr; infants, c.393_394insTA / p.Gln134TyrfsX30 - c.3507+1G>A). TEM found angulated, cleft-like lucencies, interpreted as CH crystals, in BC of each SS. No CS or mouse had such lucencies. In both infant SS, BC marked for MRP2 but not MDR3. In the woman and all CS, BC marked for both antigens.
Conclusions: With *ABCB4* mutation, even when IS finds MDR3, CH crystals may be seen in BC on TEM. (CH excretion rates are lower in mouse than in man. Different bile composition likely underlies disparate TEM findings.) When *ABCB4* disease is a consideration, liver biopsy specimens should be handled to permit TEM.

1449 Degree of Plasma Cell Infiltration in Liver Explants of Patients Undergoing Transplantation for HCV Is Predictive of the Development of *De Novo* Autoimmune Hepatitis

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Background: *De novo* autoimmune hepatitis (DNAH) is increasingly recognized post-liver transplantation (LT). Whether this is a variant of rejection, a true *de novo* hepatitis or part of an overlap syndrome is unknown. Predictive factors for development of DNAH might allow earlier recognition and treatment. True histological overlap syndromes between HCV and autoimmune hepatitis (AH) are rare. We investigated whether HCV patients developing DNAH post-LT had an overlap syndrome prior to LT.
Design: A free text search of the pathology database was performed (1994-2006) using the key words "liver allograft" and "plasma cells". We cross-referenced the data with names of all patients undergoing LT for HCV. The diagnosis of HCV was confirmed via presence of detectable viral load. We selected control cases based on year of LT and time to development of histological DNAH. H&E and trichrome slides from explanted native livers and subsequent biopsies were blindly reviewed by 2 liver pathologists. A modified Scheuer classification was used to grade the inflammation. Severity of plasma cell infiltrate was based on the proportion of plasma cells in the inflammatory infiltrate as follows: 0 none, 1 occasional plasma cells, 2 10-30%, 3 >30% often occurring in sheets; 5 random areas with dense inflammatory infiltrates were scored in the native liver and the highest score was used.
Results: We gathered 39 patients with DNAH (average age at LT: 52, range 34-70) and 35 control patients (average age at LT: 54, range 33-68). The M:F ratio was reduced in the DNAH group (24M:15F) compared to the control group (29M:6F; $p<0.05$). Explants from 16/39 (41%) DNAH patients showed a plasma cell score of 3 while only 5/35 (14%) explants from control patients had a score of 3 ($p<0.05$). No cases in either group had a score of 0. There was no significant difference in the number of cases with a score of 2 or more (DNAH: 26/39 and control: 20/35; $p=0.4$) There was no significant difference in inflammatory activity between control and DNAH patients.
Conclusions: Small numbers of plasma cells may be present in HCV cirrhosis. However, significant plasma cell infiltration in the native liver of HCV patients undergoing LT may be predictive for the development of *de novo* AH. This may be evidence for a pre-existing overlap syndrome between HCV and AH and may guide follow-up.

1450 KOC, TTF-1 and CDX2 Discriminate Small Cell Carcinoma from Carcinoid and Pancreatic Endocrine Tumor Metastasized to the Liver

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Background: Discrimination among small cell carcinoma (SCC), gastrointestinal carcinoid (GIC) and pancreatic endocrine tumor (PET) metastasized to the liver can be challenging when the biopsy is small and crushed, and clinical history is lacking. Immunostains for CDX2, TTF-1 and NESP55 have been used to aid the distinction with some success. We recently reported expression of K homology domain containing protein overexpressed in cancer (KOC) in SCCs but not in carcinoids. Here we assessed the usefulness of KOC in segregating metastatic SCCs from GICs and PETs in the liver.
Design: 27 SCCs resected or biopsied from the liver included 17 from the lungs, 4 from extrapulmonary sites (2 colon, 1 gallbladder and 1 stomach) and 6 unknown. 18 metastatic GICs and 16 metastatic PETs were also included in this study. Immunostains were performed using antibodies against CDX2, TTF-1, NESP55 and KOC. Nuclear (CDX2 and TTF-1), cytoplasmic (NESP55) and both cytoplasmic and membranous (KOC) stains were considered positive. Staining intensity was graded as weak, moderate or strong. A p value of <0.05, as determined by two-tailed Fisher exact test, was considered statistically significant.
Results: 24 (89%) SCCs showed strong KOC staining in >60% of the tumor cells. 18 (67%) SCCs were strongly and diffusely positive for TTF-1 and no signal was detected in the remaining 9 including 4 extrapulmonary SCCs. Among TTF-1- negative SCCs, 7 showed strong and diffuse KOC expression. All 16 PETs were positive for KOC with 12 (75%) showing a diffuse and moderate to strong staining pattern. Only 3 (17%) GICs showed a variable degree of KOC staining ($p<0.01$; in comparison with SCCs and PETs). TTF-1 was not detected in PETs and GICs. CDX2 was variably expressed in 17 (94%) GICs and 6 (38%) PETs. All SCCs were negative for CDX2 except for 1 of colonic origin showing positivity. Variable NESP55 expression was detected in 5 PETs.
Conclusions: Metastatic SCCs and PETs in the liver express KOC more frequently than GICs whereas GICs express CDX2 more frequently. TTF-1 is specific for SCCs, particularly for lung origin. A combination of KOC, CDX2 and TTF-1 provides a immunostaining panel more useful in the segregation among SCC, GIC and PET in a liver metastasis.

1451 Smad4 Overexpression in Mixed Hepatocellular-Cholangiocarcinoma (HCC-CC) Confirms Its Close Relation to Hepatocellular Carcinoma

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Background: Transforming growth factor beta pathway plays an important role in the development of hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC). Smad4 is a key factor for signal transmission of this pathway to the nucleus. Overexpression of smad4 has been found in approximately 50% HCCs, whereas roughly 50% CCs have loss of expression. It is unknown whether dysregulation of Smad4 protein occurs in mixed HCC-CC, an uncommon hepatic malignancy with distinct pathologic features but clinically similar to HCC.
Design: Cases of mixed HCC-CC diagnosed on hepatectomy or biopsy specimen without neoadjuvant therapy from 1995 to 2006 were identified from the pathology database. Four-micron thick paraffin-embedded tissue sections were immunostained for Smad4, CK19, and Hepar-1(Santa Cruz Biotechnology, CA). The staining intensity was scored as 0 (negative), 1+ (weak), 2+ (moderate) and 3 (strong). Sections with more than 10% of cells with at least 1+ posivitiy were considered as positive. Overexpression was defined as increased intensity of staining compared to that on the normal hepatocyte or duct epithelium.
Results: Eighteen cases of mixed HCC-CCs from 18 patients (men:women 13:5) with an average age of 58 years (range 40-73) were identified. The HCC and CC components were confirmed by morphology in combination with immunostains for biliary marker CK19 and Hepar-1. Thirteen of 18 cases had morphologically distinct HCC and CC components. All of the above cases showed overexpression of Smad4 in either cytoplasm (7/13, 54%) or both cytoplasm and nucleus (6/13, 46%) in the HCC component, and 12 cases had overexpression in cytoplasm (4/13, 31%) or both cytoplasm and nucleus (8/13, 62%) in the CC component. Three of the above cases had decreased expression in nucleus while cytoplasmic expression was increased or unchanged in the CC component. There were 5 of 18 cases with a transitional HCC-CC morphology. All 5 cases showed overexpression of Smad4 in both cytoplasm and nucleus (4/5, 80%) or nucleus only (1/5, 20%).
Conclusions: Smad4 protein is overexpressed in the vast majority of mixed HCC-CCs. This may reflect abnormal phosphorylation of Smad4 protein resulting in accumulation of Smad4 in the cytoplasm or nucleus. The findings suggest that the carcinogenesis of mixed HCC-CC may be more closely related to HCC than CC, supporting the current clinical consideration that mixed HCC-CC should be classified as HCC.

1452 VEGFr2 Expression in Hepatocellular Carcinoma and Adjacent Cirrhotic Liver

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Background: Hepatocellular carcinomas (HCC) are highly vascular tumors, and in these tumors angiogenesis is mediated at least in part by vascular-derived endothelial growth factor (VEGF). VEGF is highly expressed in HCC and mediates its angiogenic effects through multiple receptors including VEGF receptor 2 (VEGFr2, KDR/FLK-1). The multiple tyrosine kinase inhibitor Sorafenib™ inactivates the VEGFr2 receptor and demonstrates anti-tumor effects in HCC, but the distribution, localization, and relative expression levels of the VEGFr2 in HCC and background non-tumorous cirrhotic liver has not been reported.
Design: 58 HCC and corresponding adjacent cirrhotic liver samples from resection or transplant specimens were studied. Immunohistochemical staining for VEGFr2 was performed. Stains were scored by estimating the percentage of positive surface area in veins, arteries, and sinusoidal lining cells in HCC and adjacent, non-tumorous cirrhotic liver.
Results: VEGFr2 expression was limited to vascular endothelial cells and sinusoidal lining cells in HCC and benign cirrhotic liver. Overall average VEGFr2 levels ((sinusoidal lining cell, artery and vein levels) were significantly higher in HCC than in background cirrhotic liver ($p<0.001$). Greater staining for VEGFr2 staining was found in sinusoidal lining cells ($p<0.001$), arteries ($p<0.001$), and veins ($p=0.004$) of HCC as compared to cirrhotic liver. Furthermore, tumor VEGFr2 levels correlate with VEGFr2 levels in the corresponding adjacent cirrhotic liver sample levels (rho 0.79, 95% CI 0.62, 0.95, $p<0.001$); as receptor levels in cirrhosis increased, so did levels in the tumor. This correlation was seen in all sites of VEGFr2 expression (sinusoidal lining cells, vein endothelial cells, arterial endothelial cells), but was strongest for sinusoidal lining cells (rho 0.81, 95% CI 0.66, 0.97, $p<0.001$).
Conclusions: VEGFr2 is more highly expressed in HCC than in adjacent cirrhotic liver, although the receptor is found in both locations. The presence of VEGFr2 in background non-tumorous cirrhotic liver suggests that targeted VEGFr2 inhibitors may be useful in preventing tumor development in the setting of cirrhosis and in preventing HCC recurrence. The positive relationship between VEGFr2 expression levels in HCC with corresponding cirrhotic liver suggests that biopsies of background cirrhotic may predict tumor VEGFr2 expression levels and possibly tumor response to targeted VEGFr2 inhibitor therapy. The relationship between VEGFr2 expression and therapeutic response, however, is unknown and warrants further study.

1453 Increased Frequency of Central Venulitis after Liver Transplantation for Primary Biliary Cirrhosis

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Background: Central venulitis (CV) can occur in the allograft liver, but the significance of this finding for graft function and outcome is not well understood. The aim of this study is to establish the frequency of CV occurrence after liver transplantation (LT) for primary biliary cirrhosis (PBC) and its association with other clinical variables.

Design: All patients who underwent LT for PBC at our center from 2000-2006 and a control group of NASH patients matched by age, gender, and date of LT were identified. All post-LT biopsies done in subjects and controls were reviewed and characterized using the Banff schema. ACR was sub-classified as portal-based ACR features without CV (P-ACR) or portal-based ACR with CV (P-ACR+CV). Isolated CV (ICV) and centrilobular inflammation (CI) were also noted. Clinical, biochemical, surgical and vascular data were abstracted and analyzed. Comparisons between ACR, P-ACR, P-ACR+CV, ICV, and CI in PBC patients versus controls were made while controlling for other clinical variables.

Results: 25 patients who underwent LT for PBC and 23 matched controls were identified. 2 PBC patients died within 3 months of LT and were excluded. No significant differences between PBC patients and controls were demonstrated with respect to length of follow-up, ischemia time, anastomosis type, immunosuppression, and cytomegalovirus viremia. ACR was diagnosed in 52% of PBC subjects and 35% of controls (p=0.23). Differences in CV and CI frequency were noted between groups.

Comparison of histological findings in 23 patients after LT for PBC and 23 controls

	Post-OLT for PBC (n=23)	Post-OLT for NASH (n=23)	p value
P-ACR	4/23 (17%)	6/23 (26%)	0.47
P-ACR + CV	8/23 (35%)	2/23 (9%)	0.03
CV with or without P-ACR	11/23 (48%)	3/23 (13%)	0.008
ICV	3/21 (14%)	1/19 (5%)	0.3
CI	16/21 (76%)	6/19 (32%)	0.004

CV and CI were more common in PBC patients than controls. By multivariate analysis, the association between pre-LT PBC diagnosis and CV overall or with concurrent portal-based ACR was independent of other variables including hepatic artery flow, piggyback anastomosis, and immunosuppressive regimen.

Conclusions: Post-LT CV is more common in PBC patients than in NASH controls. In most cases CV was associated with the presence of portal features of ACR. These findings suggest PBC patients may have more severe rejection or unique biologic mechanisms of rejection such that the central veins are more affected than in NASH patients.

1454 Superior Mesenteric Vein/Portal Vein Invasion Is Associated with Poor Survival in Patients with Stage II Pancreatic Ductal Carcinoma Who Received Pre-Operative Chemoradiation

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Background: With recent advances in pancreatic imaging and surgical technique, patients who have pancreatic ductal carcinoma (PDC) with short-segment occlusion of the superior mesenteric vein/portal vein (SMV/PV) confluence and a suitable option for vascular reconstruction but no involvement of superior mesenteric artery or celiac axis, are considered borderline candidates for resection. However, the significance of SMV/PV involvement by PDC in patients who underwent pancreaticoduodenectomy is unclear. The purpose of this study was to evaluate the prognostic value of SMV/PV involvement in patients with stage II PDC who received chemoradiation.

Design: A total of 155 consecutive patients with stage II PDC who received preopertative chemoradiation and underwent pancreaticoduodenectomy at our institution between January 1, 1999 and December 31, 2004 were retrospectively analyzed. The H&E stained slides from all cases were reviewed. The SMV/PV involvement was defined microscopically as direct tumor invasion into the lumen of the vein, vein wall or adventitia (perivascular soft tissue ≤1.0 mm from media of the vein with fibrosis extending to the media). Statistical analyses were performed using SPSS software (version 12.0; SPSS, Chicago, IL) and survival was evaluated by Cox regression analysis.

Results: Among the 155 patients (54 stage IIA and 101 stage IIB), 45 patients had SMV/PV resection and 32 of them showed tumor involvement of SMV/PV (71%). The median disease-free survival (DFS) for patients with SMV/PV involvement by tumor was 8.9 ± 1.7 months compared to 14.4 ± 3.7 months for those patients without SMV/PV involvement or without SMV/PV resection (P=0.005), and overall survival (OS) was 21.2 ± 2.3 months compared to 28.5 ± 3.6 months, respectively (P=0.004). There were no differences in DFS or OS between the group of patients who underwent SMV/PV resection but with no microscopic involvement of SMV/PV and patients who did not have SMV/PV resection. In multivariate analyses, SMV/PV involvement (p=0.05 and p=0.011) and positive lymph node status (p=0.014 and p=0.031) correlated with DFS and OS respectively independent of T stage and differentiation.

Conclusions: The involvement of SMV/PV by pancreatic carcinoma is an independent prognostic factor and is associated with worse prognosis in patients with stage II PDC who received preoperative chemoradiation.

Neuropathology

1455 Primary Peripheral T-Cell Lymphomas of the Central Nervous System: Immunological Results and Molecular Approach to Diagnosis

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Background: Primary central nervous system lymphomas (PCNSL) are an uncommon extranodal lymphoma that involves the brain, leptomeninges, eyes or spinal cord in the absence of systemic disease. The majority of the PCNSL are diffuse large B-cell lymphomas, however, peripheral T-cell lymphomas (PTCLs) have been rarely reported. It is difficult to differentiate PTCL from a reactive T-cell process, especially when small T-cells predominate. Since there are no immunohistochemical markers for T-cell monoclonality, gene rarrangement analysis can be useful to make the diagnosis.

Design: Patient demographics (age, sex) and disease characteristics (location, histological type and treatment) were collected and hematoxylin and eosin stained sections were reviewed on 5 patients with T-PCNSL. Immunohistochemical stains for CD20, CD3, CD5, CD4, CD7, CD8, CD2, TIA-1 and Granzyme B; and in situ hybridization (ISH) for Epstein Barr virus (EBER) were performed on paraffin sections. T-cell receptor (TCR) gamma gene rearrangement analysis was performed by PCR with capillary electrophoresis.

Results: The majority of the PTCLs were located in the frontal lobe. The morphology showed a striking angiocentric pattern. Four of five tumors consisted of small to intermediate sized cells with round to irregular nuclei and scant cytoplasm. All cases were CD3 positive and three of four cases with sufficient tissue showed an abnormal T-cell antigen pattern (double negative CD4/CD8, double positive CD4/CD8, or loss of CD5; see Table).

Case details

	Case 1	Case 2	Case 3	Case 4	Case 5
Age (yrs)/ Sex	46/F	71/M	44/F	36/M	53/M
Location of Tumor	Frontal lobe	Frontal lobe	Frontal lobe	Cerebellum	Meninges, Retina
Symptoms	Headache	Memory loss	Headache	Multiple sclerosis	Neurological deficit
Histology	SI	SI	SI	SI	LC
CD20	-	-	-	-	-
CD3	+	+	+	+	+
CD5	+	-	-	+	+
CD4	-	+	+	+	ND
CD7	-	+	+	+	ND
CD8	-	+	+	-	ND
CD2	+	+	+	+	ND
EBER	-	-	-	-	ND
TCR rearrangement	Clonal	Clonal	Clonal	Clonal	ND
TIA-1	-	+	+	-	ND
Granzyme B	-	+	+	-	ND

M: Male; F: Female; SI: Small/Intermediate; LC: Large cell; ND: not done

Four of four cases studied showed a monoclonal T-cell receptor gamma gene rearrangement.

Conclusions: Since primary CNS-PTCL may mimick inflammatory, infectious and vascular diseases, morphology and immunohistochemical studies may be insufficient for diagnosis. TCR gamma gene rearrangement analysis assists in confirming the diagnosis of lymphoma.

1456 Use of CD10, CA9, and RCC To Distinguish between Clear Cell Meningioma and Metastatic Clear Cell Renal Cell Carcinoma

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Background: Clear cell meningioma can be difficult to distinguish from metastatic clear cell renal cell carcinoma by standard light microscopy. Differentiation between these two entities is essential in determining patient treatment and prognosis. The purpose of this study is to evaluate the utility of immunomarkers CA9, CD10, and RCC in differentiating between clear cell meningioma and metastatic clear cell renal cell carcinoma.

Design: A retrospective review of immunostaining with CA9, CD10, and RCC in 18 clear cell meningiomas compared with clear cell renal cell carcinomas (n=39).

Results: Eighteen patients (9 males, 9 females) with clear cell meningiomas were studied. The age range of this group at the time of surgery was 16 to 86 years (mean 58.5 years). The most common tumor sites included the meninges overlying the frontal lobe (n=7), the clinoidal region (n=2), and the cavernous sinus (n=2). Clear cell meningioma showed some immunoexpression of CA9 and CD10 in 38% (n=7) and 28% (n=5) of tumors, respectively. Most cases with positive staining showed < 5% positivity. No immunoexpression of RCC by clear cell meningioma was observed. Thirty-nine patients (27 males, 12 females) with either primary or metastatic clear cell renal cell carcinoma made up the renal cell carcinoma component study group. The age range of the patients at the time of surgery was 39 to 87 years (mean 63.5 years). Seventeen of the tumors were metastases and 22 were primary. The clear cell renal cell carcinomas stained positively for CA9, CD10, and RCC in 100%, 96%, and 42% of cases, respectively, with the majority of cases showing diffuse expression.

Conclusions: The immunohistochemical stains CA9, CD10, and RCC are useful in differentiating clear cell meningioma from metastatic clear cell renal cell carcinoma. The immunohistochemical marker RCC is not expressed in clear cell meningioma in this study, while CA9 and CD10 have limited expression in a minority of meningiomas. The combination of these stains may prove to be a useful panel when confronted with this differential diagnosis.

1457 Evaluating Interobserver Reliability in a Classification of Malformations of Cortical Development (MCD)

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Background: Malformations of cortical development (MCD) are a well-recognized cause of intractable epilepsy. A simplified histologic classification of MCD has been proposed which divides MCD into mild MCD and focal cortical dysplasia (FCD) types I and II (Palmini A et al. Terminology and classification of the cortical dysplasias.