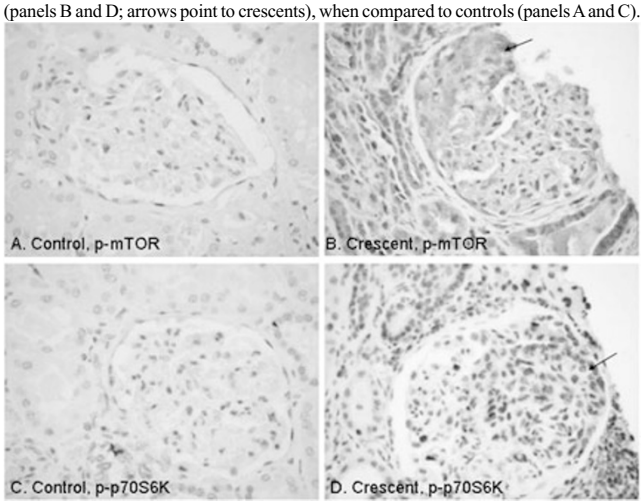
glomeruli was 53.3%. A total of 22 out of 25 (88%) CGN showed upregulated nuclear staining of p-p70S6K and cytoplasmic staining of p-mTOR in the crescentic epithelium (panels B and D; arrows point to crescents), when compared to controls (panels A and C).



The 3 negative stained cases included 1 with fibrocellular crescents, 1 without crescents in deep sections, and 1 without glomeruli in the deep section. On PAS stained sections, upregulated unclear p-p70S6K was further confirmed in the cellular crescents.

Conclusions: In renal biopsies with various kidney diseases, a prominent upregulation of mTOR pathway signals in glomerular visceral/parietal epithelium and renal tubular epithelium was observed in all glomerulonephritis cases, indicating an increased proliferative activity in many types of glomerulonephritis. Upregulated p-mTOR and p-p70S6K in the crescentic components raise the possibility of using mTOR inhibitors as an alterative treatment in CGN.

1715 Stem/Progenitor Cell Marker CD133 Identifies Glomerular and Tubular Injury in Human Renal Biopsies

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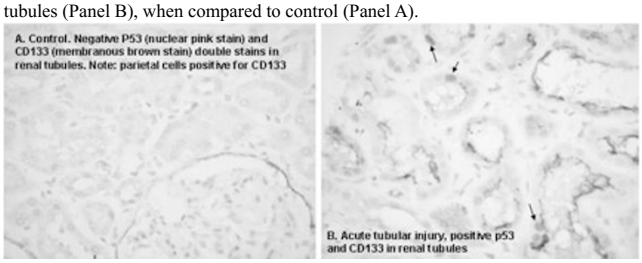
Background: CD133, a stem/progenitor cell marker, expresses in parietal epithelial cells (PEC) of normal glomeruli and crescentic glomerulonephritis by immunofluorescent methods. This study was to investigate immunohistochemical (IHC) expression of CD133 in glomerular and tubules injury from renal biopsies.

Design: There were 16 control unremarkable renal sections (from nephrectomy for renal tumors). The acute kidney injury (AKI) group was composed of 63 native renal biopsies, with 9 primary acute tubular necrosis (ATN), 5 tubulointerstitial disease, 13 primary crescentic glomerulonephritis (GN), 14 thrombotic microangiopathy, 9 collapsing GN/HIV nephropathy, 6 biopsies with variants of monoclonal light chain nephropathy and 8 pediatric renal biopsies. They were all immunohistochemically stained for CD133 (monoclonal AC133 antibody at 1:50 dilution and a polyclonal CD133 antibody 1:500).

Results: Stains using two types of CD133 antibodies demonstrated a similar pattern of staining in normal and injured renal tissue. CD133 staining in normal PEC and crescents was confirmed as reported. In the normal kidney, there was minimal or rare CD133 staining in tubular epithelium. But in the injured proximal tubules, a gradient CD133 expression along luminal membranes was present from 1+ to 3+ (63 out of 63 cases), depending on the extent of injury. With AKI, many distal-nephron tubular cells expressed CD133 along luminal membranes as well. Double stains for CD133 (brown membranous stain) and p53 (pink nuclear stain) showed co-expression in injured renal tubules (Panel B), when compared to control (Panel A).



Furthermore, the tubular expression of CD133 was significantly correlated with serum creatinine levels.

Conclusions: We confirmed CD133 expression in normal PEC and crescents of CGN as shown previously. Importantly, the tubular upregulation of CD133 along luminal membranes implies that renal "progenitor-like" cells exist along tubular epithelium for tubular regeneration and the tubular injury can significantly contribute to AKI even in conventional "glomerular disorders". Finally, the IHC staining for CD133 can be used for confirming lesions in glomeruli and renal tubules of renal biopsies, better than any marker we tested.

1716 Beneficial Effects of Exogenous Thymosin ß4 on Late Stage Tubulointerstitial Fibrosis

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Background: Previously we showed that thymosin β4 (Tβ4), a G-actin sequestering protein, is remarkably increased in the obstructed kidney in the unilateral ureteral obstruction (UUO) model of tubulointerstitial fibrosis. Ac-SDKP, the degradation product of Tβ4 generated by prolyl oligopeptidase (POP), has anti-fibrotic effects. Moreover, we found that inhibition of POP increased Tβ4 levels, decreased Ac-SDKP levels, and exacerbated the early stage of fibrosis in obstructed kidneys. We have now investigated the long-term effects of thymosin β4 on fibrosis.

Design: Male C57BL/6 mice underwent UUO with treatments as follows, and were sacrificed at day 14: UUO without treatment, UUO+Tβ4 (150μg, i.p. q 3 d), UUO+combination (POP inhibitor, S17092, 40mg/kg/d, by gavage and Tβ4), and UUO+Ac-SDKP (1.6 mg/kg/d, delivered by minipump).

Results: Tubulointerstitial fibrosis assessed by Sirius red morphometric analysis was significantly higher in mice treated with POP inhibitor+Tβ4 combination compared to untreated UUO (3.37±0.21 vs. 2.92±0.09%, $p < 0.05$). Fibrosis was dramatically reduced by Ac-SDKP, and surprisingly, also by Tβ4 alone (Ac-SDKP 2.56±0.13; Tβ4 1.90±0.04%, both $p<0.05$ vs. untreated). We next examined POP enzyme activity and Ac-SDKP levels in the obstructed kidneys. POP activity was significantly decreased in the UUO kidneys of mice with combination treatment compared to untreated UUO kidneys (29% of levels in untreated UUO, $p<0.05$). Neither Ac-SDKP nor Tβ4 administration changed POP activity. Ac-SDKP concentration was significantly reduced by combination treatment, but was dramatically increased by Ac-SDKP or Tβ4 administration vs. untreated UUO (combination, 51%; Ac-SDKP, 199%; Tβ4, 149% of untreated UUO, all $p<0.05$ in treated vs. untreated).

Conclusions: Our study suggests that long-term exogenous Tβ4 administration may have net anti-fibrotic effects in UUO kidneys. The data demonstrate that this beneficial effect could be due to the combined actions of Tβ4 and its downstream product, Ac-SDKP. The potential beneficial effects of Tβ4 need further investigation.

Liver

1717 Fatty Liver Contributes to Hepatocarcinogenesis in Non-Cirrhotic Livers

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Background: While many specific etiologies of hepatocellular carcinoma (HCC) are well known, such as viral hepatitis and cirrhosis, the exact mechanisms of hepatocarcinogenesis remain unclear, particularly in non-cirrhotic livers. Diabetes and obesity have been established as independent risk factors for HCC. Oxidative stress has also been suggested in carcinogenesis. As steatosis is a common hepatic manifestation of these conditions, we studied the prevalence of hepatic steatosis in non-cirrhotic livers with HCC.

Design: All resected HCC cases arising in non-cirrhotic livers from 2001-2010 in 3 tertiary centers in US were searched. All resected cholangiocarcinoma (CC) cases in non-cirrhotic livers from 2001-2010 were used as control. After excluding all etiologies such as hepatitis B and/or C, hemochromatosis, α-1 antitrypsin deficiency, liver adenoma, etc, there were 157 HCC and 120 CC cases. Slides of liver distant from tumor were reviewed and steatosis was scored according to NASH-CRN. Tumor slides were reviewed in a subset of cases. Clinical data including metabolic profile were collated.

Results: As shown in the Table, the prevalence of significant steatosis (at least grade 1) in non-tumor (NT) liver in 85/157 (54%) HCC cases was greater than in 32/120 (26%) CC cases ($p<0.0001$). NT hepatic steatosis was associated with obesity ($p=0.003$) in HCC and with obesity ($p=0.002$) and diabetes ($p=0.04$) in CC. NT steatosis was associated with obesity ($p<0.0001$) and diabetes ($p=0.006$) in HCC and CC cases altogether. NT steatosis was not associated with age or alcohol use. In 40 HCC cases with tumor slides available, 18 showed the recently described steatohepatitic(SH)-HCC morphology, in which 16 had significant steatosis in NT liver, while 11 of the 22 without SH-HCC pattern had significant steatosis in NT liver ($p=0.016$). NT steatosis was not associated with steatosis in HCC.

Grade of steatosis in non-tumor liver

	0	1	2	3
HCC (n=157)	72 (46%)	56 (36%)	22 (14%)	7 (4%)
CC (n=120)	88 (73%)	21 (17%)	10 (8%)	1 (1%)

Conclusions: The prevalence of steatosis in NT liver was higher in HCC than in CC. The latter is similar to the general US population with hepatic steatosis. Obesity and diabetes were associated with hepatic steatosis in both HCC and CC. Hepatic steatosis was also associated with SH-HCC morphology. This multi-center and large cohort study underlines the role of hepatic steatosis and metabolic syndrome in hepatocarcinogenesis in non-cirrhotic liver.

1718 Pathological Characteristics of the Livers of Patients with Itai-Itai Disease (Chronic Cadmium Toxicity)

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Background: Itai-itai disease (IID), which is recognized in Japan as a pollution-related illness, is caused by chronic exposure to the mineral cadmium (Cd). Excess Cd accumulation in the renal proximal tubules causes renal atrophy and secondary osteomalacia. Liver failure is a well-known complication of acute Cd toxicity, but

there are no detailed reports of the liver histopathology of IID patients. In the present study, we assessed the histopathological characteristics of the livers of IID patients.
Design: Autopsy specimens from 49 IID patients and 27 patients not exposed to Cd pollution were examined. Cd concentration was measured using fresh organs. H&E staining was performed along with Berlin blue staining for detecting iron deposition and immunostaining for metallothionein (MT), which binds and detoxifies Cd, and megalin, which binds to Cd–MT complexes and leads to endocytosis.
Results: The Cd concentration in the liver was highest among all of the assessed organs from the IID patients; it was second highest after the kidney among the controls. Cd accumulation in the organs of the IID patients was 5.2 (liver), 4.1 (pancreas), 4.9 (thyroid gland), 7.3 (muscle), 3.3 (rib), 0.38 (renal cortex), and 0.62 (renal medulla) times higher than that of the controls. No common pathological changes such as necroinflammation, fibrosis, or carcinogenesis were observed in the livers of the IID patients. Hemosiderosis was found in 29/49 cases. MT expression was greater in the livers of the IID patients compared with the controls, but there was no remarkable expression of megalin in either group.
Conclusions: Because the liver is a known target of acute Cd toxicity, we hypothesized that Cd specifically accumulates in the liver, resulting in chronic liver injury. However, Cd also accumulated in most of the other organs, and there was no remarkable expression of megalin in the liver. Therefore, we found that Cd accumulation is not liver-specific. Moreover, despite the high Cd concentration in the liver, no specific injury was detected, indicating that the liver can detoxify Cd. Possible reasons for these results are (1) the liver is the main MT-producing organ, and thus, it is easier to retain MT–Cd complexes here, resulting in a high level of Cd in the liver, and (2) the quantity of Cd accumulation in the liver does not easily reach a toxic level because endocytosis of Cd by megalin is not highly activated. Thus, it appears that kidney injury among IID patients is restrained by increasing the expression of MT and by inhibiting the effect of megalin.

1719 The Steatohepatitic Variant of Hepatocellular Carcinoma Is Associated with Non-Alcoholic Steatohepatitis

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Background: While individual features of non-alcoholic steatohepatitis (NASH) such as fat, inflammation, fibrosis, and ballooning degeneration with or without Mallory's hyaline, are relatively common in hepatocellular carcinoma (HCC), the combination of these features to produce changes resembling NASH within the tumor is relatively rare. This steatohepatitic variant of HCC (SH-HCC) has been described in association with chronic hepatitis C (HCV). We hypothesized that SH-HCC may also arise in the setting of NASH.
Design: We reviewed all available resection and transplant cases of HCC from the surgical pathology files of our institution from 1994-2011. Tumors were evaluated for features of NASH: steatosis, ballooning degeneration, inflammation, pericellular fibrosis, and Mallory's hyaline. Cases were classified as SH-HCC if 4 or 5 of these features were present. Clinical records were reviewed for patient age, gender, body mass index (BMI), alcohol abuse history, and etiology of underlying liver disease. Frequency distributions were evaluated by a χ^2 test.
Results: A total of 238 tumors from 236 patients were examined, including 156 explants and 80 partial hepatic resections. 114 tumors showed no features of NASH, and 55 showed 1 to 3 features. Sixty-nine (29.2%) showed 4 or 5 and were classified as SH-HCC. Clinical characteristics by HCC subtype are shown in Table 1.

Clinical Features in SH-HCC versus Standard HCC

	SH-HCC (n=69)	Standard HCC (n=169)	
Age (mean, y)	59.0	57.0	
Gender (M/F)	62/5	135/34	
BMI (mean)	28.3	28.9	
Alcohol abuse	13 (18.8%)	37 (22.0%)	NS
HCV	37 (53.6%)	99 (58.6%)	NS
NASH or cryptogenic	14 (20.3%)	15 (8.8%)	p=0.015

NS=Not statistically significant

Patient age, BMI, alcohol history, and HCV status were similar between SH-HCC and standard HCC. Fewer SH-HCC patients were women (7.3%) than standard HCC patients were (20.1%). Among SH-HCC patients, 14 (20.3%) had either cirrhosis secondary to NASH or cryptogenic cirrhosis, compared to 15 (8.8%) among patients with standard HCC (p=0.015).
Conclusions: The SH-HCC pattern is associated with underlying NASH in our population. Patients with HCV-related HCC appear no more likely to have SH-HCC than the general population of HCC patients does. These data suggest an etiologic link between NASH and a steatohepatitic phenotype in HCC that is different from the HCC variant in HCV patients. This correlation could impact treatment options and outcomes for this growing population of patients.

1720 Arterialization and Ductular Metaplasia of Centrizonal Scars in Chronic Venous Outflow Obstruction: A Frequently Misinterpreted Lesion

B Can, G Krings, L Ferrell. 19 Mayis Univ, Samsun, Turkey; Univ Calif, San Francisco.
Background: Chronic venous outflow obstruction (CVOO) is characterized by centrizonal hepatocyte ischemia with hepatocyte atrophy, necrosis, sinusoidal fibrosis and zone 3 scarring. Recent studies have shown that other conditions associated with centrizonal scarring, including steatohepatitis and alcoholic cirrhosis, often show architectural remodeling resulting in aberrant centrizonal arterialization and ductular metaplasia (DM) of hepatocytes. In our experience, centrizonal scars in CVOO may also develop arterial ingrowth and DM, but this has not been systematically evaluated. Recognition of these changes is essential to prevent misinterpretation of centrizonal lesions as portal tracts and misdiagnosis of CVOO as a biliary tract problem and/or ductopenia.
Design: Fifty-eight cases of documented CVOO were evaluated for centrizonal arteries and microvessels, DM, and fibrosis. The number of centrizonal arteries in scar was graded as 0-3 by H&E and trichrome stains. DM was semi-quantitatively scored on a scale of 0-3. Immunostains for keratin 7 (K7) and CD34 were performed (n=24) to assess for DM and hepatocyte staining as well as centrizonal arteries and microvessels, respectively. Centrizonal fibrosis was staged using the NASH CRN system. Patients with other concomitant conditions or cholestatic liver injury were excluded.
Results: Arterial ingrowth was identified in centrizonal scars in 47 (81%) of 58 cases. Arteries were common (grade 2-3) in 27/58 (46.6%). CD34+ microvessels were observed in centrizonal scars in 22 (91.7%) of 24 cases. Centrizonal DM was identified in 41 (70.7%) of 58 cases by H&E. K7 staining revealed centrizonal DM and hepatocyte intermediate staining in 20 (83.3%) and 22 (91.7%) of 24 cases, respectively. Centrizonal arteries and DM were significantly more frequent in cases with higher stage fibrosis (p=0.008 and p=0.001, respectively). Significant association was found between the presence of centrizonal arterialization and DM (p=0.01).
Conclusions: Centrizonal arterialization and DM are commonly associated with CVOO, and both correlate with the stage of fibrosis. Arterial ingrowth appears to be part of the vascular remodeling of progressive fibrosis. DM and intermediate K7 staining of hepatocytes are likely due to ischemia of hepatocytes in Zone 3 in CVOO. Recognition of these aberrant features is critical to prevent misinterpretation of central zones as portal tracts, which may otherwise result in misdiagnosis of CVOO as a biliary tract problem with or without ductopenia.

1721 Hepatic Injury in the Liver Allograft Biopsy Continues Despite Hepatitis C Viral Clearance with Interferon Treatment

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Background: Hepatitis C virus (HCV) recurrence following orthotopic liver transplantation (OLT) frequently results in progressive allograft injury, including cirrhosis and graft failure. Treatment of recurrent HCV after transplantation with interferon-based regimens has become widely accepted and has the potential to result in sustained virological response (SVR). The goal of this study is to determine what the histological outcome of SVR is in the transplanted liver.
Design: Fifty-five patients with recurrent HCV following liver transplantation and histological evidence of progressive hepatic fibrosis completed a standard course of interferon-based anti-viral therapy. Patients were categorized according to serologic response to therapy as measured by HCV RNA PCR. Progression of inflammatory activity and fibrosis was determined by histological comparison of biopsy material obtained before initiation of therapy to biopsies obtained during SVR. Biopsies were scored according to activity (0-3) and fibrosis (0-6). The presence and severity of acute and/or chronic rejection (ACR, CR) was recorded. The change in activity and fibrosis was measured over time.
Results: Thirty-one patients (56%) had undetectable serum levels of HCV RNA by PCR at the end of the therapy period, five of which serologically relapsed. Twenty-six patients (47%) achieved SVR. Twenty-four patients (44%) failed to demonstrate serologic viral clearance. Biopsy material for evaluation of progression of activity and fibrosis was available in 14/26 SVR patients (54%) and 16/24 nonresponders (67%). Time elapsed between pre-treatment and SVR biopsies ranged from 6-65 months (median 17) for the SVR group and from 3-37 months (median 8.5) for the nonresponder group. The Δactivity and Δfibrosis ranged from -0.04 to 0.036/month (median 0) and -0.033 to 0.333/month (median 0), respectively, for the SVR group and -0.3 to 0.091/month (median 0) and 0 to 0.667/month (median 0.06), respectively for the nonresponder group. There were 4 (29%) episodes of ACR in the SVR group and 3 (19%) in the nonresponder group. CR developed in 2 (14%) patients from the SVR group and 3 (19%) from the nonresponder group.
Conclusions: We conclude that hepatitic injury in the liver allograft persists despite SVR in response to interferon-based therapy. Additionally, there is no statistically significant difference in the Δactivity and in the Δfibrosis/time between biopsies from SVR and nonresponder liver allografts. Moreover, SVR may be associated with a mild increase in episodes of ACR.

1722 Epistein-Barr Virus Associated Primary Intrahepatic Lymphoepithelioma-Like Cholangiocarcinoma

AWH Chan, JHM Tong, MYM Sung, PBS Lai, K To. The Chinese University of Hong Kong.
Background: Lymphoepithelioma-like cholangiocarcinoma (LELCC) is a rare variant of intrahepatic cholangiocarcinoma (IHCC). Only 14 cases have been reported in the literature, and about 10 of them (70%) were associated with EBV. DNA hypermethylation is frequently observed in both EBV-associated carcinoma and IHCC, but has not been documented in LELCC yet.
Design: Seven cases of LELCC and 11 cases of IHCC in stage I were evaluated. Immunostaining for CK19, HepPar-1, EBV LMP-1, ER, PR, CD21 and p53, and in situ hybridization of EBER were performed. Methylation statuses of APC, ATM, DAP-kinase, E-cadherin, GSTP1, HIC1, HLTF, MGMT, MLH1, P14, P15, P16, RASSF1A, SOC-1, DLEC1, RARB, CRBPI, CRBPIV, ATF5 and DAB2 were analyzed by methylation-specific polymerase chain reaction.
Results: All 7 patients of LELCC were exclusively female and had stage I diseases. LELCC had a significantly better 2-year overall survival (100% vs 52.8%, p=0.011). All LELLC were composed of glandular component and 3 of them were mixed with undifferentiated compinent. Both glandular and undifferentiated components showed variable expression of CK19 but not HepPar-1, ER, PR and CD21. All were positive for EBER ISH but negative for EBV LMP-1. The methylation frequency of each locus in LELCC varied from 5.6% to 94.4%. The methylation index ranged from 0.30 to 0.55 and 0.15 to 0.50 in LELCC and IHCC, respectively. The mean methylation index of

LELCC was statistically higher than that of IHCC (0.429 vs 0.291, p=0.016). Regardless of the EBV status, APC, E-cadherin, GSTP1, RASSF1A and RARB were methylated in more than 35% of all studied cases. Only CRBPI (85.7% vs 9.1%, p=0.002) and CRBPIV (85.7% vs 0%, p<0.001) showed statistically higher methylation frequencies in LELCC than IHCC. There was no statistical association among survival data, tumor size, histological grade, immunophenotype and methylation status in LELCC and IHCC.
Conclusions: A cohort of seven cases of EBV-associated LELCC was reported, which is the largest case series in the literature. EBV-associated LELCC is a distinctive variant of IHCC, characterized by marked female predominance, more favorable overall survival, and high frequent DNA hypermethylation. High incidence of CRBPI and CRBPIV methylation in LELCC suggested the disruption of the retinoic acid signaling pathway is important in its carcinogenesis.

1723 High Fat/Cholesterol Diet Leads to Accumulation of an Electron-Dense Lamellar Material in Pig Hepatocytes

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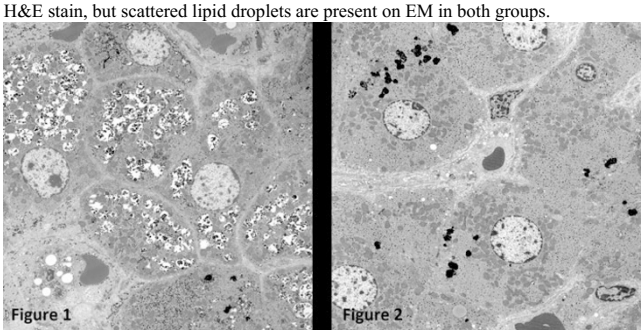
Background: Nonalcoholic fatty liver disease, one of the most common chronic liver diseases in humans, is clinically categorized into simple steatosis and nonalcoholic steatohepatitis (NASH). While simple steatosis is believed to be reversible, NASH progresses to cirrhosis over a variable period of time. Diagnosis of NASH is based on the presence of ballooned hepatocytes, which are thought to signify cellular damage by as yet undefined factors. We describe the ultrastructural changes in hepatocytes of Ossabaw swine, which represent a large animal model of metabolic syndrome and nutritionally-induced NASH.
Design: Ossabaw swine were fed a modified atherogenic diet (NASH diet group; n=5) consisting of 16.5% calories from protein, 20% calories from fructose, 46% calories from fat (admixture of hydrogenated soybean oil, coconut oil, and lard), and 2% cholesterol or standard chow (control group; n=6) for 24 weeks. Differences between the two groups were evaluated on H&E sections and electron microscopy (EM).
Results: Hepatocytes in pigs fed the NASH diet show accumulation of electron-dense material arranged as whorled lamellar structures (n=5) (fig 1). This material is present within membrane-bound vesicles, consistent with secondary lysosomes. Hepatocytes in the control group do not contain similar material (n=0) (fig 2). All but one pig fed the NASH diet show variable numbers of enlarged, pale staining hepatocytes on H&E stain (n=4). This change is not seen in the control group (n=0). Steatosis is not seen on H&E stain, but scattered lipid droplets are present on EM in both groups.



Conclusions: Pigs fed the NASH diet accumulate an abnormal material in their hepatocytes, which is reminiscent of membranous profiles observed in a variety of conditions such as phospholipidosis and the phospholipid storage disease, Niemann-Pick. This similarity raises the possibility that the accumulated material represents phospholipids, possibly arising from damaged cellular or subcellular membranes. This may in turn reflect the effects of increased hepatocyte lipid loading. The finding of similar material in human NASH may pave the way for more accurate identification of ballooned hepatocytes.

1724 Temporal Association of Infection to Ductular Cholestasis in Liver Biopsies

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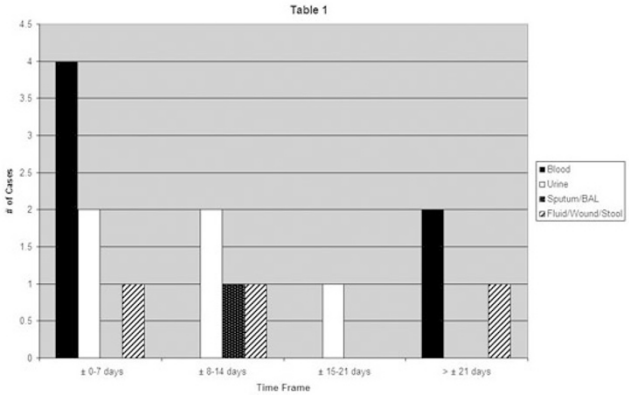
Background: Severe infections and septicemia are known to result in ductular (cholangiolar) cholestasis which is characterized by dilated ductules containing bile casts present at edges of portal tracts. However, it is not known how long these changes persist after infection or if they precede clinical or laboratory detection of infection.
Design: Liver biopsies showing ductular cholestasis were identified by a free text search of the pathology database for various combinations of the words "cholangiolar" and "ductular" with "cholestasis" and "casts". Cases with another prominent pathological process identified histologically (i.e., acetaminophen toxicity, neoplasm, allograft rejection) were excluded. Electronic medical records were examined for evidence of infection, source of positive culture, cultured organism and timing of positive culture from biopsy.
Results: 14 cases were identified; there were 7 males and 7 females ranging from 22 to 76 years (mean 50 years). The biopsies were performed for elevated liver enzymes. Underlying clinical diagnoses included solid organ transplantation (5 cases), idiopathic liver failure (2 cases), malignancy (3 cases) and miscellaneous conditions (4 cases). 12 of the 14 cases had either clinically suspected or laboratory identified infections, from 1 day to 2 months after biopsy; 1 case did not have laboratory evidence of infection, but clinically died from septic shock 3 days after the biopsy. 1 case showed no clinical or laboratory evidence of infection.



Positive cultures were most commonly obtained from blood (6/12 cases) followed closely by urine (5/12 cases). Gram negative rods were the most common microorganism, led by *E. coli*. Other organisms isolated included; *E. cloacae, P. aeruginosa, K. pneumoniae, E. faecium, C. tropicalis,* and *C. glabrata*.
Conclusions: Ductular cholestasis was associated with clinical or laboratory evidence of infection in 12/14 cases (86%). However, this evidence could be demonstrated within 7 days of biopsy in only 7/12 (58%) cases. Although the remaining 5 cases had evidence of infection, the causal relationship in these cases is doubtful when positive cultures are detected many days after the biopsy.

1725 Fibrosis in the Time Zero Liver Allograft Biopsy Predicts Decreased Long Term Graft Survival: Implications for Liver Allograft Allocation and Mechanisms of Failure

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Background: Time zero liver allograft biopsies are routinely performed at the time of liver transplantation. We have previously shown that several features in these biopsies are predictive of outcomes within the first three months (Human Pathology 27(10):1077-1084, 1996). The goal of this study was to determine which features in the time zero biopsy were predictive of long term outcomes.
Design: From 545 adult patients undergoing liver transplantation between 2005-2010 at our institution, 101 patients were randomly selected for re-review of their time zero liver biopsies for % macrovesicular steatosis, fibrosis (0[none], 0.5[minimal], 1[moderate portal expansion], 2[septate], 3[bridging], 4[cirrhosis]; trichrome stain), inflammation (0-3), apoptotic and mitotic indices, and hepatocyte swelling. Kaplan Meier survival curves were constructed for each variable at 3 months, 1 year, and 3 years, with the endpoint "graft survival" defined as patient death or re-transplantation. We also compared the slide re-reviews with the original time zero pathology report.
Results: Only the feature of fibrosis significantly predicted reduced survival, from 87.5% to 63.6% (p=.044) at 3 years post transplantation. Patients with at most "mild" fibrosis had a mean survival of 110 days longer than those grafts with more significant fibrosis. Our cohort consisted of 83% men and 17% women with median age 53 years (19-73), 50% of whom were transplanted for cirrhosis due to hepatitis C. The median cold ischemic time of 5.3 hours is short compared to UNOS of 8.2. Our cohort composition for fibrosis was 0 = 85%, minimal = 2%, 1 = 10%, and 2 = 1%. For steatosis, 84% had < 10%, 12% had 10-30%, and 4% had > 30%. Compared to the original pathology report, the re-review of the % steatosis and degree of fibrosis did not significantly differ.
Conclusions: That pre-existent fibrosis predicts decreased long term survival post liver transplantation may have implications in the method and criteria for evaluating and deciding on the use of livers for engraftment. As well, our results imply that fibrogenic processes already set in motion prior to liver engraftment have long term biologic effects.

1726 Small Vessel Hepatic Hemangioma, an Atypical Hemangioma Variant in Adult Liver

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Background: We have encountered rare atypical vascular lesions in the liver, not previously described, which are composed entirely of small vessels, often with an infiltrative border, and which may mimic well-differentiated angiosarcoma on biopsies. This study aims to further characterize these lesions.
Design: Small vessel hemangioma (SVH) samples (n=8) ranged from core biopsies (n=3) to partial resections (n=4) to autopsy (n=1). When possible, CD31, CD34, Ki-67, and p53 immunostains were performed. Proliferative index (PI) represents percentage of Ki-67 positive tumor nuclei/HPF. Benign vascular liver tumors (cavernous hemangiomas (CH), n=10) as well as malignant vascular liver tumors (epithelioid hemangioendotheliomas (EHE), n=8, and angiosarcomas (AS), n=7)) were similarly evaluated.
Results: The average age for SVH patients was 49 (range 34-83, n=7), mostly men (6/7). All cases (n=7) represented an incidental finding detected by imaging or at autopsy, usually a single lesion. The average size was 1.8 cm (range 0.7-4 cm, n=6). One patient had a similar splenic lesion. Recognition as hemangioma was not possible on imaging in at least one case. No patients, for which data is available, have died of disease (12 months maximum follow up). Lesions were uniformly composed of small vessels, usually with an infiltrative border and entrapped bile ducts, and occasionally

with extramedullary hematopoiesis. Cytologic atypia and mitotic activity were not present. All cases were positive for CD31 and CD34 (n=5), negative for p53 (n=4), and overall had a low PI (average PI=4%, range 0-10%, n=6). By comparison CH did not show Ki-67 labeling (PI=0%, n=10), EHE had a similar average PI (6%, range 0-12%, n=8), and AS had a higher average PI (32%, range 15-50%, n=7). CH also did not demonstrate p53 staining, but a subset of EHE and AS had strong nuclear staining (EHE 3/8; AS 2/7).

Conclusions: Small vessel hepatic hemangiomas are a distinct rare variant of hemangioma encountered in adults. These lesions are typically identified as an incidental finding. The infiltrative border may raise concern for well-differentiated angiosarcoma on biopsy. The PI is above that which is typically seen in benign cavernous hemangioma and the lack of strong nuclear p53 staining argues against consideration as angiosarcoma. Given limited outcome data, this lesion may be one of uncertain malignant potential and we recommend excision if possible.

1727 Clinical Associations of Ito Cell Hyperplasia

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Background: Ito cell hyperplasia (ICH) characterized by H&E identifiable, prominent peri-sinusoidal hepatic stellate cells laden with lipid droplets, has been principally attributed to hypervitaminosis A. Although sporadic reports of ICH in other liver diseases such as chronic biliary disease exist, clinical associations of this entity have not been studied in detail. We aimed to examine the clinical conditions, other than hypervitaminosis A, in which ICH occurs.

Design: By searching our pathology database, we identified 34 patients (15 male and 19 female) ranging in age between 4 and 77 years old (median: 56) whose liver biopsy demonstrated ICH (between 1997 and 2011). We re-examined the routine sections (H&E, trichrome, PAS-D, and iron) of the original and any follow up liver biopsies to confirm the presence ICH and evaluate any additional histologic changes. Clinical, radiologic, and laboratory data (including serum vitamin A levels) were reviewed and their relationship to ICH was examined.

Results: ICH occurred in 24 native and 10 transplant liver biopsies. All patients used medications and (82%) were multi-medicated. 99 different drugs were used at the time of the liver biopsy, most frequently, prednisone, proton pump inhibitors and furosemide. In 30 (of 34) patients, there was no clinical or laboratory evidence of hypervitaminosis A. Based on clinical, laboratory and histologic features, the biopsies were classified as follows:

1. Native livers
a. Drug induced hepatitis (n=6), histologically pan-acinar (n=4) or reactive (n=2). Niacin was the most frequent inciting agent (3 of 6)
b. Chronic liver disease (n=10): chronic hepatitis C (n=4), autoimmune hepatitis (n=3), steatohepatitis (n=1), chronic biliary disease (n=1), and portal venopathy (n=1).
c. Non-specific reactive hepatitis (n=4); related to systemic illness (n=2) or inflammatory gastrointestinal disorder (n=2).
d. Hypervitaminosis A (n=4)
2. Transplanted livers
a. Bile outflow impairment (n=5)
b. No significant abnormalities in routine allograft biopsy (n=4)
c. Resolving acute cellular rejection (n=1)

Follow-up biopsies were available in 5 patients at intervals ranging from 15 days to 16 months; in 2 patients, progression of fibrosis was observed in the setting of chronic biliary disease, and the remaining 3 patients showed no fibrosis.

Conclusions: ICH is an uncommon and relatively nonspecific finding that most commonly occurs in multi-medicated patients, often in the absence of hypervitaminosis A. It occurs in association with drug toxicity such as Niacin, chronic liver disease, systemic illness or inflammatory disorders of the gastrointestinal tract, as well as in the post liver transplant setting.

1728 Hepatic Glycogenosis in Children with Nonalcoholic Fatty Liver Disease (NAFLD): A Different Disease Than Glycogenic Hepatopathy

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Background: Diffuse hepatic glycogenosis is the dominant histological finding in glycogenic hepatopathy, a condition sometimes seen with poorly controlled diabetes mellitus type 1 (DM1). A similar histologic feature (glassy, pale cytoplasmic accumulations on H&E stain) has been noted in pediatric NAFLD liver biopsies which lacked the typical clinicopathological pattern of glycogenic hepatopathy. Our aim was to further characterize the clinical and histological correlations of this finding.

Design: Liver biopsies blindly reviewed centrally by the pathology committee of the NASH CRN were scored as having either no, focal (involving <50% of hepatocytes) or diffuse (>50%) glycogenosis. These data were analyzed for associations with other histologic features and clinical characteristics including age, sex, diabetes status, aminotransferases, alkaline phosphatase, fasting glucose, fasting insulin and HOMA-IR.

Results: Biopsies were reviewed in 139 children (≤18 years of age). Mean age was 12.6 years (range 5-18) and 72.7% were male. Glycogenosis was focal in 19 (13.7%) and diffuse in 31 (22.3%). Glycogenosis was associated with low steatosis grade (p=0.016), large droplet macrovesicular steatosis (p=0.014), lower NAFLD Activity Score (NAS) (p = 0.049) and increased non-hepatocellular iron (p=0.038). No associations were identified with ballooning, Mallory-Denk bodies, megamitochondria, lobular inflammation, portal inflammation or fibrosis. The one patient with DM1 had diffuse glycogenosis. There were no associations with aminotransferase levels, fasting glucose, fasting insulin or HOMA-IR.

Conclusions: In pediatric NAFLD, hepatic glycogenosis is found in more than 33% of biopsies. It is associated with lesser degrees of steatosis and a lower NAS. There are no associations with markers of NASH severity, fibrosis or insulin resistance. Hepatic glycogenosis should not be over interpreted as a true glycogenic hepatopathy.

1729 Subjective Pathologic Estimates of Viable Tumor in Ablated Hepatocellular Carcinomas (HCC) Are Adequate for Routine Practice and for Radiology/Pathology Correlation Studies

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Background: Ablation of HCC is employed to induce complete tumor necrosis. Incomplete necrosis predicts HCC recurrence and may affect transplant options. Clinicians rely on radiologists and pathologists to accurately asses treatment response. Studies examining radiology/pathology correlation in determining complete response have shown poor agreement, however, the pathologic methods used to assess viable tumor (VT) are often not uniform or are poorly described. We compared subjective and objective measurements of the percentage of viable tumor (PVT) in ablated HCCs to determine if subjective estimates are reliable.

Design: Two pathologists independently reviewed gross images and H&E stained slides for 23 ablated HCCs. The livers were serially sectioned and ablation cavities (AC) were measured in three dimensions. VT was defined as non-necrotic tumor within or contiguous with an AC. Each observer subjectively estimated PVT after gross and microscopic review (eyeball estimate). Each observer then measured individual areas of VT foci using formulas based on their shapes and added them to yield a composite viable tumor area (CVTA) for each AC. The CVTA was converted to a composite viable tumor volume (CVTV) based on the spherical nature of the AC. The CVTV was divided by the volume of the ablation cavity as determined by gross measurements, yielding an objective estimate of PVT. Means and standard deviations were calculated for all estimates and paired sample t-tests were conducted examining the inter and intraobserver agreements.

Results: The 2 pathologists had strong interobserver agreement of PVT using both the subjective [means: 25.7%(KM), 25.3%(JH); p=0.81] and objective methods [means 21.5%(KM), 21.6%(JH); p=0.75]. The intraobserver agreement between subjective and objective methods was less strong [p=0.09 (Km), p=0.05 (JH)] without a clear trend. Data analysis revealed 5 outliers with poor intraobserver agreement. On review, less than 1 section per cm of AC was submitted for each of these 5 cases. The paired sample t-tests were re-calculated without the poorly sampled ACs and yielded stronger intraobserver agreement between the two methods [p=0.38(KM), p=0.69(JH)].

Conclusions: This study demonstrates that a subjective assessment of PVT correlates well with measured PVT. It therefore validates the subjective pathologic assessment of HCC ACs as an accurate measure of the PVT. It also emphasizes the importance of adequate sampling of the AC to ensure accurate estimates.

1730 IgG4 Reaction in Biliary Cancers – Signification and Mechanism

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Background: IgG4-related diseases are characterized by the increased serum IgG4 and the infiltration of marked IgG4+ plasma cells, but malignant tumors mimicking IgG4-related diseases have been reported. IL-10 is an important regulatory cytokine associated with the differentiation of IgG4-producing plasma cells. In this study, the signification and mechanism of IgG4 reaction were investigated in extrahepatic biliary cancers.

Design: Immunohistochemistry of IgG4, CD8, HLA-DR, CD80, CD86, and Foxp3 was performed using 68 cases of surgical specimens from patients with 39 gallbladder cancers, 21 common bile duct cancers, and 8 cancers of the papilla of Vater. Moreover, using two cultured cell lines originated from human cholangiocarcinoma, molecular analysis for Foxp3 and IL-10 was performed.

Results: 1. Association between IgG4 reaction and immune surveillnce. Immunohistochemistry for IgG4 and CD8 was performed using 68 cases of biliary cancers including common bile duct cancers, gallbladder cancers, and cancers of the papilla of Vater. Although IgG4+ cells were few or none in the majority of cases, ≥10 and ≥50 cells/high power field [HPF] were found in 37% and 6% of cases, respectively. In the cases with over 10 IgG4+ cells/HPF, cytotoxic CD8+ T cells were few within tumor, suggesting the evasion of immune surveillance. 2. Biliary cancers as non-professional antigen presenting cells (APCs). Immunohistochemistry revealed that MHC class II molecule (HLA-DR)-positive and costimulatory molecules (CD80 and CD86)-negative tumors indicating the possible induction of IL-10-producing regulatory T cells (anergy T cells) were found in 42%, and the number of IgG4+ cells in these cases was higher than those of the others. 3. Biliary cancers as Foxp3+ regulatory cells. The antibody recognizing N terminus of Foxp3 highlighted cancer cells as well as Treg cells and Foxp3+ cancers had more numerous IgG4+ cells than Foxp3- ones. In vitro study using cultured human cholangiocarcinoma cell lines demonstrated the presence of splicing variant of Foxp3 mRNA and the expression of IL-10 mRNA, suggesting the regulatory function of Foxp3+ cancer cells.

Conclusions: Extrahepatic biliary cancers are often accompanied by the significant infiltration of IgG4+ cells and IgG4 reaction in biliary cancers might be associated with the evasion of the tumor-related immune surveillance. Moreover, cancer cells could play a role of regulatory functions by themselves directly and indirectly via a production of IL-10.

1731 Mucinous Cystic Neoplasms of the Gallbladder: A Clinicopathological and Immunohistochemical Study

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Background: Mucinous cystic neoplasms **(**MCN**)** of the hepatobiliary tree are rare tumors, but even rarer are MCN arising from the gallbladder (GB). Most are lined by a single layer of either gastric or pancreatobiliary epithelium with ovarian-type stroma. This study aims to characterize the clinical, histological and immunohistochemical (IHC) profile of GB MCN and compare them with intrahepatic (IH) MCN.

Design: The pathology database from 1995-2011 was searched; clinical data was gathered from medical records. Histological assessment was performed; IHC using antibodies to CK7, CK19, MUC2, MUC5AC and MUC6 was performed. Immunoreactivity was scored: 0 negative; 1 focal; 2 <50% cells + staining; 3 >50% strong + staining. Clinicopathological features and mucin profile were compared with 12 previously studied IH MCN.

Results: Six GB MCN of 16,638 GB specimens were found (0.03%). Four of 6 were female, mean age 72 years (range 55-90). All were incidentally found during unrelated surgical procedures (3 resection for malignant hepatic tumors, 2 colectomy, 1 harvesting for cadaveric donation). Five of 6 (83%) were located in the fundus; 1 in the GB neck; 4/6 (67%) were of the multilocular type, and 2 with combined multilocular and papillary architecture. Size ranged from 0.9-5cm (ave 1.8cm). Pure pancreatobiliary lining epithelium was found in 4/6 and mixed gastric and pancreatobiliary in 2/6; ovarian type stroma was seen in all cases. No epithelial dysplasia or carcinoma was found. The pancreatobiliary-type epithelium had 3+ staining for CK7, CK19 and MUC6; MUC2 was 1+ in 3 (50%). All cases were reactive to MUC5AC but with variable staining (1+ in 4, 3+ in 2). The gastric foveolar epithelium in the two mixed cases was 3+ for MUC5AC and MUC6, and 1+ for MUC2. Table 1 compares GB and IH MCN at our institution.

Table 1

	GB MCN	IIH MCN
M:F	1:2	1:11
Age	72yrs(55-90)	45yrs(33-62)
Size	1.8cm(0.9-5)	8cm(1-29)
Epithelium	67% pancreatobiliary 33% mixed	33% pancreatobiliary 59% mixed 8% gastric
Ovarian stroma	100%	100%
MUC2	50%	8%
MUC5AC	100%	50%
MUC6	100%	42%

Conclusions: GB MCN are very rare tumors. Similar to IH MCN, they occur mostly in females. GB MCN, however are found in older patients. Lesions are smaller and are often incidentally found. Pure pancreatobiliary epithelium is the main lining in most cases whereas IH MCN are mainly mixed gastric and pancreatobiliary. GB MCN display a strong pancreatobiliary IHC phenotype as displayed by their reactivity for CK7 and CK19. The mucin expression of the tumor differs from IH MCN, suggesting that the phenotype of the cells is different from those of the liver.

1732 Morphologic Features Predict Prognosis in Intrahepatic Cholangiocarcinoma

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Background: Intrahepatic cholangiocarcinoma (ICC) is an uncommon malignancy with a poor prognosis. The incidence has increased in the recent years. Definitve prognostic factors for patient survival have not been clearly identified.

Design: To determine significant factors associated with outcome, retrospective review of pathological findings of the resected tumors and uninvolved liver were performed in patients from a single tertiary hospital. Tumors were grouped into 5 patterns: ductal, papillary, mucinous, sclerosing and mixed (each pattern ≥ 25% or with HCC component). The tumor was also evaluated for differentiation, lymphovascular invasion (LVI), perineural invasion, size and T(tumor) classification. Steatosis, fibrosis, inflammation and ductal atypia were graded in the uninvolved liver.

Results: The study group was 23 ICC patients resected for ICC from 2001 to 2011 (female:13; male: 10; age: 66.8 ± 11.6 years) with complete clinical and survival data. The overall survival after resection was 1.7 ± 0.5 years. Statistical analysis showed that the survival was significantly associated with tumor stage, LVI and mixed pattern. Survival was not significantly related to the tumor size, differentiation, perineural invasion, or hepatic parenchyma with adjacent bile duct atypia, fibrosis or steatosis (p>0.05). The mean survival for the patients with mixed pattern ICC was significantly lower than those with a solitary pattern (p<0.05).

Univariate Analysis of Survival and Tumor Pathologic Characterstics in ICC Patients

Classification	subclassification	Patient numbers (n)	Survival (years)	p value
Tumor size	<5 cm	8	2.8 ±1.2	0.21
	≥5 cm	15	1.1±0.3	
LVI	Absent	10	3.0±1.0	<0.01*
	Present	13	0.6±0.1	
Perineural invasion	Absent	14	1.1±0.3	0.13
	Present	9	2.6±1.1	
Tumor staging	I	2	7.7±3.0	<0.01*
	II	10	1.1±0.2	
	III	7	1.3±0.6	
	IV	3	1.0±0.1	
Tumor pattern	Mixed	14	0.7±0.1	<0.05*
	Solitary	9	2.9±1.0	

Univariate Analysis of Survival and Morphologic Characteristics of Uninvolved Liver

Classification	Sub-classification	Number of patients (n)	Survival years	p value
Liver fibrosis	Absent	4	0.75±0.21	0.43
	Stage I	6	1.5 ± 0.9	
	Stage II	5	4.1± 0.9	
	Stage III	5	2.0± 0.8	
	Stage IV	3	0.8± 0.5	
Liver steatosis	Absent	14	1.7 ± 0.7	0.91
	Mild-Severe	9	1.6± 0.5	
Adjacent duct atypia	Absent	11	1.2± 0.4	0.39
	Present	11	2.1 ± 0.9	

Conclusions: The current findings suggest that tumor staging, LVI, and tumor pattern are independent prognostic factors for ICC after surgery. The mixed pattern ICC has a worse prognosis than a solitary tumor pattern. The backgound inflammation, fibrosis stage and steatosis amount does not appear to affect outcome in our study.

1733 Reassessment of Steatosis in Donor Liver Biopsies

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Background: Steatosis appears to be an important factor in determining liver allograft suitability and predicting graft function. 30% is a commonly cited steatosis content cutoff for graft use. Studies of livers containing >30% fat have shown mixed results, and controversy exists as to the appropriate assessment of steatosis and viability of steatotic grafts.

Design: We reviewed all donor liver (time-zero and frozen) biopsies performed at our institution from 01/2005 to 10/2010 to assess for macrovesicular, microvesicular, small droplet and total (sum of all types) steatosis. Chart review was performed and graft steatosis was correlated with donor and recipient factors. Outcome measures include primary nonfunction (PNF), graft survival and patient survival. JMP software was used for statistical analyses.

Results: 530 biopsied grafts were used for transplantation. Biopsies were classified by percentage of parenchymal volume involved by various types of steatosis into two groups (<30% and >= 30%). No biopsies contained >5% pure microvesicular steatosis. Small droplet (data not shown) and macrovesicular steatosis were not associated with unfavorable outcomes (Table 1). Total steatosis >=30% was associated with increased PNF in our patient population (table 2). Four grafts showed >= 60% steatosis; one had PNF.

Macrovesicular steatosis

	<30% (n=511)	>=30% (n=19)	p value
Age (yrs)	54.2	58	0.07
Donor age (yrs)	41.2	48.6	0.03
PNF	14 (2.7)	2 (2.5)	0.11
Graft survival at 1 mo	480 (93.9)	16 (84.2)	0.12
Graft survival at 1 yr	430 (84.1)	16 (84.2)	1.0
Pt survival at 1 mo	491 (96.1)	18 (94.7)	0.54
Pt survival at 1 yr	441 (86.3)	16 (84.2)	0.8

Mean for age and N (%) for others.

Total steatosis

	<30% (n=478)	>=30% (n=52)	p value
Age (yrs)	54.2	55.3	0.52
Donor age (yrs)	41	46.3	0.015
PNF	11 (2.3)	5 (9.8)	0.014
Graft survival at 1 mo	451 (94.4)	45 (86.5)	0.07
Graft survival at 1 yr	405 (84.7)	41 (78.9)	0.32
Pt survival at 1 mo	460 (96.2)	49 (94.2)	0.45
Pt survival at 1 yr	414 (86.6)	43 (82.7)	0.4

Mean for age and N (%) for others.

Conclusions: There was no increase in PNF or decrease in graft or patient survival seen with grafts containing >=30% small droplet or macrovesicular steatosis. Total steatosis >=30% was associated with a higher rate of PNF, but did not affect patient or longer term graft survivals. Few patients received >60% steatotic grafts. Further studies are needed to identify additional factors that predict outcome in steatotic grafts.

1734 Steatohepatitis: Distinct Pathway(s) to Cirrhosis?

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Background: Steatohepatitis is divided into two major categories: alcohol induced fatty liver disease (AFLD) and non-alcohol induced fatty liver disease (NAFLD) which are essentially indistinguishable on H&E. Recent literature proposes that AFLD and NAFLD result from distinct mechanisms of injury although the details are essentially unknown. Lipid accumulation and subsequent injury related to excessive alcohol use is believed to result, in part, from intrahepatic lipid synthesis following an ER stress response. In contrast, accumulation of lipids in NAFLD has been reported to result from both the diet as well as made within the liver. Several proteins within these various pathways have been implicated to be differentially expressed in AFLD and NAFLD; however, much of the literature is overlapping and unclear. Delineating the molecular details of these pathways would be of great clinical benefit as distinguishing AFLD from NAFLD is currently not possible by pure morphologic features.

Design: Pathology databases from the University of Chicago were searched from 2010-2011 for biopsies and explants containing a final diagnosis of steatohepatitis. All grades and stages were included. Clinical history provided within the pathology report was used to choose 10 AFLD and 10 NAFLD cases. Immunohistochemistry on FFPE tissue for SAFB and SREBP-1 (transcription factors involved in regulation of fatty acid synthesis), CD36 (fatty acid translocase which brings circulating fatty acids into the hepatocyte) and FAS (fatty acid synthesis) was performed and graded independently for intensity (0-3) and location by 2 pathologists. Scores of 2 and 3 were considered positive.

Results: Immunostaining for SREBP-1 showed significantly increased nuclear expression in 70% of AFLD vs 30% of NAFLD liver specimens (p=0.02). This increased nuclear expression was independent of steatohepatitis grade, stage or percentage of steatosis. Antibodies against SAFB, FAS and CD36 did not distinguish AFLD from NAFLD.

Conclusions: Significantly increased nuclear expression of transcription factor SREBP-1 in ALFD is independent of grade and stage. This may reflect distinct molecular pathways responsible for ongoing injury leading to cirrhosis in steatohepatitis. Further investigation into the mechanisms behind increased SREBP-1 expression may unveil differential expression of additional proteins within these pathways. These findings may provide the initial step to definitively distinguish AFLD from NAFLD.

1735 Hepatocellular Carcinomas Arising in Adenomas: Similar Immunohistochemical and Cytogenetic Features in Adenoma and Hepatocellular Carcinoma Portions of the Tumor

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Background: Well-differentiated hepatocellular carcinoma (WD-HCC) in non-cirrhotic liver can show morphological features similar to hepatocellular adenoma (HCA). In rare instances, HCC can arise in the setting of HCA. This study compares the immunohistochemical and cytogenetic features of the HCA-like and HCC portions of these tumors.

Design: Immunohistochemistry for beta-catenin (bC), glutamine synthetase (GS), serum amyloid associated protein (SAA), glypican-3 (GPC) and heat shock protein 70 (HSP70) was done in 11 cases of HCC arising in HCA in non-cirrhotic liver. Tumors with nuclear bC and/or diffuse GS were considered bC-activated. Fluorescence in situ hybridization (FISH) was done in 9 cases for gains of chromosomes 1, 8 and c-myc.

Results: There were 7 men (33-75 yrs) and 4 women (30-65 years). Focal atypical morphological features like small cell change and focal pseudoacinar architecture were seen in HCA-like areas in 7 (64%) cases. HCA-like areas showed features of inflammatory HCA in 7 (64%) cases; 4 of these also showed SAA+ in the HCC portion. bC activation was present in majority of HCA-like areas, while positive HSP70 and chromosomal gains on FISH were seen in significant minority of HCA-like areas (see table). Of the 6 cases in which HCA-like area showed bC-activation, 3 (50%) showed chromosomal gains on FISH and 2 showed positive HSP70. All patients with chromosomal gains in HCA-like portions were men. For HCA-like areas that were bC-activated and HSP70 positive, 67% and 25% respectively were in men. Of the 2 women <50 years of age, bC activation was absent in the HCA-like regions, while HSP70 was positive in 1 case.

	βcatenin	GS diffuse	SAA	GPC	HSP70	Abnormal FISH
HCA-like area	37	55	64	0	40	44
HCC area	46	64	37	0	70	67

Numbers reflect percentages

Conclusions: HCA-like portion of most cases of HCC arising in HCA shows features typically seen in borderline tumors or HCC such as focal morphological abnormalities, bC-activation, HSP70 expression and chromosomal gains. HCA-like areas in these tumors, especially in men and older women, may represent an extremely well-differentiated variant of HCC, while the morphologically recognizable HCC portion represents a relatively higher grade component of the tumor.

SK,JPG-equal first authors

1736 E Antigen Expression in Hepatitis B Virus Infection Is Associated with Core Antigen Expression and More Severe Disease Activity

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Background: Patients with chronic hepatitis B (HBV) infection may have detectable serum E antigen (HBeAg), reflecting actively replicating virus, whereas absence of HBeAg denotes an inactive carrier state or infection with precore-mutated virus. Data regarding the relationships between HBeAg status, immunohistochemical staining patterns for hepatitis B core (HBcAg) and surface (HBsAg) antigens, and severity of liver disease are conflicting. The purpose of this study was to evaluate the associations, if any, between HBeAg status and these other parameters.

Design: 103 patients with chronic HBV-related hepatitis and known HBeAg status were included in the study. Liver biopsies from all patients were assessed for inflammation and fibrosis using a four-tiered system. Each case was subjected to immunostains for HBcAg and HBsAg. The Wilcoxon Ranked Sum Test and Spearman Rank Correlation Test were used to examine associations between histologic (inflammatory activity, fibrosis, percentage of HBcAg- and HBsAg-positive hepatocytes), clinical (age, sex, ethnicity, genotype), and serologic (HBeAg, HBV DNA titers, ALT) findings.

Results: 38 patients (M/F=11/8, mean age: 34 years) were HBeAg+, 18 (47%) of whom were born outside the U.S., and 65 patients (M/F=31/34, mean age: 44 years) were HBeAg-, including 48 (74%) born outside the U.S. and 17 (26%) with precore mutation. Overall, HBeAg+ patients had more hepatic inflammation ($p<0.05$) and fibrosis ($p<0.05$), more extensive HBcAg staining ($p<0.05$), and higher HBV DNA titers ($p<0.05$) and ALT levels ($p<0.05$) than HBeAg- patients. Extent of nuclear and cytoplasmic HBcAg staining reflected increased HBV DNA titers in both groups, denoting active viral replication. However, extent of HBcAg staining was also associated with inflammation ($p<0.05$) and fibrosis ($p<0.05$) in HBeAg- patients. The extent of HBsAg staining was not associated with any histologic or clinical parameters in either group.

Conclusions: HBeAg is a marker of viral replication and usually reflects immune tolerance, clearance, or reactivation in chronically infected patients. Thus, HBeAg+ patients usually have more active HBV-related liver disease than HBeAg- patients, although HBcAg and HBsAg are not helpful in their evaluation. These markers may be helpful in HBeAg negative cases because their staining patterns can be used to subclassify patients. Those who are HBcAg-/HBsAg+ or HBcAg-/HBsAg- are likely in the inactive carrier state, whereas those with HBcAg+ probably harbor HBV with precore mutations.

1737 Glycogenosis Is Associated with Measures of Insulin Resistance in Adults with Non-Alcoholic Fatty Liver Disease (NAFLD)

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Background: Hepatocyte glycogenosis is a change in which hepatocytes fill with glycogen and acquire a glassy, pale appearance on routine stains. Glycogenosis has been identified as a dominant change in some type 1 diabetics biopsied for elevated aminotransferase levels. In this clinical situation, glycogenosis is thought to result from poor diabetic control. We have observed it in NAFLD, but its clinical and histologic associations are unknown. We sought to identify demographic, biochemical and histologic associations with glycogenosis in adults with NAFLD.

Design: Liver biopsies were blindly reviewed by the central pathology committee of the Nonalcoholic Steatohepatitis Clinical Research Network and scored as having either no (0%), focal (involving <50% of hepatocytes) or diffuse (>50% of hepatocytes) glycogenosis. This data was analyzed for associations with other histological features, age, sex, diabetes status, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), fasting glucose, fasting insulin, and homeostatic model assessment-insulin resistance (HOMA-IR). Chi-square, Fisher's exact P, and Mann-Whitney U tests were used to determine significance as appropriate.

Results: Biopsies were available on 417 patients (mean age 50 years, 61.9% female). Glycogenosis was focal in 107 and diffuse in 78 biopsies. Glycogenosis was associated with female sex, less steatosis, more ballooning injury, megamitochondria and a lower NAFLD Activity Score (NAS), all $p < 0.05$. Diffuse glycogenosis was seen more often in biopsies with definite NASH or without evidence of NAFLD (steatosis < 5%). There was no association with age, the diagnosis of type 2 diabetes, ALT, AST, AP or fasting glucose. Patients with any degree of glycogenosis had higher fasting insulin levels (26.8 vs 23.2 μU/mL, p=0.006) and HOMA-IR (7.9 vs 6.7, p=0.008).

Conclusions: Although not associated with type 2 diabetes, elevated glucose or aminotransferase levels, glycogenosis in patients biopsied for NAFLD was associated with higher fasting insulin and HOMA-IR levels.

1738 Ki-67 and p53 Expression as an Immunohistochemical Tool in the Differential Diagnosis of Benign and Malignant Biliary Lesions

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Background: Benign biliary lesions may morphologically and immunophenotypically mimic well-differentiated cholangiocarcinoma (CC), leading to diagnostic dilemmas, especially in small biopsies. High proliferative index (PI) as determined by Ki-67 staining and p53 expression are often considered as evidence in support of malignancy in various diagnostic settings. However, systematic assessment of the expression of these markers and evidence-based evaluation of their potential utility in differentiating benign from malignant biliary lesions has not been reported.

Design: Immunostains for Ki-67 and p53 were performed on resection and biopsy specimens of CC (n=8), biliary hamartoma (BH; n=11), biliary adenoma (BA; n=5), and parenchymal extinction (PE; n=10). PI and p53 staining was calculated by quantifying the highest percentage of positive tumor nuclei per section. Staining intensity was scored as 0 (negative), 1+ (weak), or 2+ (strong).

Results: The PI as determined by Ki-67 staining ranged from 10.1-39.9% with a mean of 19.4 ± 9.7% in CC, which was significantly increased compared to BH (1.0±1.5%), BA (0.7±1.0%) and PE (0.4±0.3%). All CC but no benign lesions demonstrated a PI exceeding 10%. Total p53 expression (5.9±5.4%) varied from 0.2-17.7% in CC and was significantly increased with respect to BH (1.2±1.9%), BA (0.0±0.1%) and PE (2.9±5.2%). Strong (2+) p53 staining was present in 7 (88%) of 8 CC cases but only 1 (4%) of 26 benign lesions ($p<0.0001$).

Ki-67 and p53 staining in biliary lesions

	BH (n=11)	BA (n=5)	CC (n=8)	PE (n=10)	p-value (CC vs BA+BH)	p-value (CC vs benign)
Ki-67 (%)	1.0±1.5	0.7±1.0	19.4±9.7	0.4±0.3	<0.0001	<0.0001
p53 (%)	1.2±1.9	0.0±0.1	5.9±5.4	2.9±5.2	0.002	0.008
Cases with p53 (2+)	1/11 (09%)	0/5 (0%)	7/8 (88%)	0/10 (10%)	0.0002	<0.0001

Conclusions: CC display a significantly increased proliferative index in contrast to BA, BH, and PE, and a threshold of 10% positive Ki-67 nuclei differentiated CC from benign lesions. Similarly, p53 expression is significantly increased in CC versus benign lesions, and strong p53 staining may be seen in nearly all CC cases but only rarely in benign lesions and can be used as a specific marker for malignancy in this context. Addition of Ki-67 and p53 to the immunohistochemical panel may therefore facilitate distinction of CC from benign biliary lesions.

1739 Long-Term Outcome of Patients Transplanted for Primary Biliary Cirrhosis: Follow-Up > 60 Months

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Background: Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease that leads to cirrhosis requiring liver transplantation. While posttransplant survival in these patients is excellent, there is a 9-35% reported recurrence. Histologic hallmark of recurrent PBC (rPBC) is the presence of granulomatous duct injury or "florid duct

lesion". Chronic inflammation other than the florid duct lesion has also been described. The aim of this study was to characterize the clinicopathologic findings and outcome in patients transplanted for PBC at our institution, with histologic and clinical follow-up of at least 60 mo.

Design: In this retrospective review study patients were identified from the transplant database, and met the following criteria: 1) pretransplant diagnosis of PBC 2) were positive for antimitochondrial antibody, and 3) had biopsy follow-up of at least 60 mo. Patients with overlap syndrome (AIH/PBC) were excluded. Electronic medical records and relevant pathology slides were reviewed on all patients (MK). All patients received ongoing standard immunosuppression and medical treatment based on clinicopathologic findings. Histologic review included assessment for inflammation, changes of recurrent disease, fibrosis and other salient findings. rPBC was diagnosed histologically if there was presence of granulomatous duct injury. Histologic grading and staging were performed on a scale of 0-4.

Results: A total of 20 patients transplanted between 1998-2006 met the study criteria. Patient age ranged from 38-76yrs (mean 61yrs). Two patients had hepatocellular carcinoma in the explant. Two patients had failed allografts due to chronic rejection (3mo) and biliary necrosis (4mo). Histologically rPBC was present in 8 (40%) patients, first identified 8-92 mo after transplant (mean 44 mo). All cases were low stage (0-2) at the time of diagnosis. Histologic follow-up after diagnosis ranged from 0-93 mo (mean 52), and disease progression was seen in only one patient (from stage 1 to 2). 3 patients who did not have rPBC had unexplained hepatitis of grade 2 or more; with follow-up of 65-94 mo one developed stage 3 fibrosis. 19 study patients are alive with clinically stable graft function (61-153 mo, mean 100 mo); one patient died of unrelated cause at 132 months.

Conclusions: 1) rPBC occurred in 40% of our patients. 2) Recurrent disease has relatively slow histologic progression 3) Post-transplant unexplained hepatitis may represent autoimmune hepatitis in these patients. 4) Patients transplanted for PBC have an excellent clinical outcome.

1740 Type II Ground Glass Hepatocytes Are Strongly Associated with Fibrosis Stage and Hepatocellular Carcinoma

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Background: Ground glass hepatocytes (GGH) can be found in chronic hepatitis B (HBV) infection and contain surface antigens (HBs) in the endoplasmic reticulum. Two types of GGH have been described: an inclusion-like pattern in hepatocytes with a scattered distribution throughout the lobules (type I) and a peripheral staining of hepatocytes with distinct clustering of cells in the lobules (type II). Studies have shown type II GGH contain mutants with deletions over pre-S2 region in HBV genome and may represent preneoplastic lesions of HBV-related hepatocellular carcinoma (HCC). In fact, it has been shown small islands of clonal hepatocytes with integrated HBV can expand. The aim of this study is to investigate the distribution patterns of HBs in GGH on immunohistochemistry (IHC), and their association with the development of HCC.

Design: Explanted or resected livers from 45 HBV patients, including 20 non-HCC (9 cirrhotic and 11 non-cirrhotic) and 25 HCC (11 cirrhotic and 14 non-cirrhotic) were retrieved. Formalin fixed sections were immunostained for HBs. The patterns of HBs expression on IHC were examined and the clustering of the type II GGH in liver was semiquantitatively assessed: 1: 1-25%; 2: 26-49%; 3:50-74%; 4:>75%. Histology of the HCCs was examined. Ishak scores were obtained in the non-tumor livers.

Results: In the non-tumor livers, type I GGH was not associated with HCC and were present in 18/20 (90%) of cases without HCC and in 22/25 (88%) of cases with HCC(p=0.8). In contrast, type II GGH were present in 11/22 (50%) of cases without HCC and in 21/25 (84%) of cases with HCC (p=0.03). Further supporting a link with HCC, type II GGH predominated in cases with HCC: they outnumbered type I GGH in 30% of cases without HCC vs 95% of cases with HCC (p=0.0002). Further, type II GGH were in clusters in 9/20 (45%) non-HCC and 21/25 (84%) HCC cases (p=0.005). Also, livers harboring HCC had more extensive type II GGH clusters (p=0.01). Type II GGH in clusters were more common in advanced than in early fibrosis (stages 3-6 vs 0-2, p=0.04) but there was no difference when comparing low vs high Ishak inflammation grade (0-3 vs 4 and above, p=1). The presence of type II GGH did not show an association with any specific histologic type of HCC. Type II GGH was not associated with HBe Ag, anti-HBe, or HBV DNA levels.

Conclusions: While type I GGH do not appear to be associated with HCC, type II GGH are strongly associated with HCC: they are more frequent and have a distinctive clustering pattern in cases with HCC.

1741 Bile Salt Export Pump (BSEP): A Sensitive and Specific Marker of Hepatocytic Differentiation in Liver Tumors

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Background: Bile salt export pump (BSEP) is an ATP-dependent transporter of bile acids expressed exclusively in hepatocyte canaliculi. BSEP has been shown to be expressed in some hepatocellular carcinomas (HCC) as well as in benign hepatocytic tumors, but its immunohistochemical (IHC) expression has not been evaluated as a diagnostic tool for the evaluation of neoplasms in liver.

Design: Tissue microarrays including: 48 HCC, 18 hepatocellular adenomas (HCA), 41 cholangiocarcinomas (ChCa), 23 metastatatic lesions (METS) (15 adenocarcinomas [colon-12; upper GI-1; pancreas-1; and breast-1]; and 8 neuroendocrine tumors [pancreas-2; small bowel-4; unknown-2]) were stained for BSEP (mouse monoclonal IgG2a, clone F-6, 1:100, Santa Cruz) and compared to other IHC markers used in this context, including CD10, HepPar-1, and glypican-3. BSEP expression was also assessed in normal tissue including breast, thyroid, lung, small intestinal, pancreas, thymus, breast, squamous mucosa, tonsil, lymph node, fat, testicle, prostate, and kidney.

Results: BSEP staining by immunohistochemistry was easy to interpret with no background staining.

Distinct canalicular expression was seen in all normal livers and HCA but was absent in all other normal tissue samples.

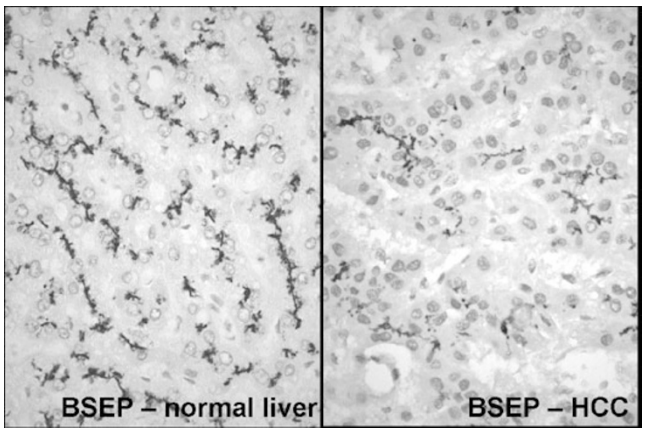
Staining in HCC was canalicular in most cases (33 of 43 positive cases).

3 of 5 HepPar-1-negative cases were positive for BSEP (all poorly differentiated HCCs).

Frequency of positivity in malignant tumors

	HCC (n=48)	ChCA(n=41)	METS (n=23)
BSEP	43 (89.6%)	0	0
CD10 (Canalicular only)	25 (52%)	0	0
HepPar-1	43 (89.6%)	2 (4.8%)	2 (8.6%)*
Glypican-3	30 (62.5%)	1 (2.4%)	1 (4.3%)^

*Positivity seen in metastatic adenocarcinoma from duodenum and neuroendocrine carcinoma from unknown primary and ^metastatic neuroendocrine carcinoma from pancreas.



Conclusions: BSEP was 100% specific and 90% sensitive for identification of hepatocytic tumors.

High specificity obviates the need for a "canalicular" pattern, which greatly limits the utility of other canalicular markers.

CD10 was 100% specific when definite canalicular staining was identified; however, membranous/cytoplasmic staining was observed in 41% of ChCa and 35% of METS and is often difficult to interpret.

BSEP is valuable in the immunohistochemical evaluation of tumors in liver.

1742 Oncogenic SULF2 Protein Expression Is Associated with Pathogenesis of Cirrhosis and Hepatobiliary Carcinoma

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Background: Hepatocellular carcinoma (HCC) is the third leading cause of cancer death worldwide and often diagnosed at an advanced stage at which there are limited treatment options. There is a strong interest in identifying novel molecular targets for therapy of advanced HCC. Sulfatase 2 (SULF2) is a recently identified human heparin-degrading endosulfatase. We have previously reported that upregulation of tumor SULF2 mRNA desulfates cell surface heparan sulfate proteoglycans (HSPGs) leading to increased HCC cell proliferation in vitro and tumor growth in vivo, and is correlated with a rapid recurrence after surgery and short survival in patients with HCC (Lai, et al, Hepatology, 2008 and 2010). Syndecan 1 (Syn1) is one of the components of HSPGs.

Design: To further investigate the role of SULF2 in pathogenesis of cirrhosis and hepatobiliary carcinoma as well as the interaction between SULF2 and Syn 1, we measured the SULF2 mRNA/18S in 35 benign liver specimens with or without cirrhosis by Real-time RT-PCR and evaluated SULF2 and Syn1 expression in 65 benign livers, 40 HCCs and 21 cholangiocarcinomas (CCs) by immunohistochemistry.

Results: We found that SULF2 mRNA was significantly up-regulated in benign liver with cirrhosis (n=19) as compared to that in benign liver without cirrhosis (n=14) (P=0.0003). This result was also confirmed in the selected 65 benign livers (35 with cirrhosis, score 6, Ishak modified staging) by immunohistochemistry using antibody against SULF2. For HCCs and CCs, each case was scored with 3+, 2+,1+ or – for both SULF2 and Syn1. High level of SULF2 (2+ and 3+) was found in 33 (82%) HCCs and 17 (80%) CCs, and low level (1+ and -) in 7 (18%) HCCs and 4 (20%) CCs. High level of Syn1 (2+ and 3+) was found in 25 (62%) HCCs and 7 (33%) CCs, and low level (1+ and -) in 15 (38%) HCCs and 14 (67%) CCs. There was a significant negative correlation between SULF2 and Syn1 in HCCs (n=40, P=0.011), but not in CCs (n=21, P=0.490).

Conclusions: This is the first report regarding the role of SULF2 in cirrhosis and the interaction between SULF2 and Syn1 in HCCs and CCs. Oncogenic SULF2 may desulfate Syn1 in both cell surface and extracellular matrix promoting pathogenesis of cirrhosis and hepatobiliary carcinoma.

1743 Liver Steatosis Assessment: Correlations among Pathology, Radiology, Clinical Data and Automated Image Analysis Software

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Background: Assessing the degree of hepatic steatosis plays an important role in many liver diseases and during orthotopic liver transplantation. Macrovesicular steatosis is associated with post-transplant dysfunction, directly proportional to the level of steatosis.

However, estimating the degree of steatosis is prone to both intra- and inter-observer variability among pathologists. Image analysis and computerized algorithms have been used with varying degrees of success.

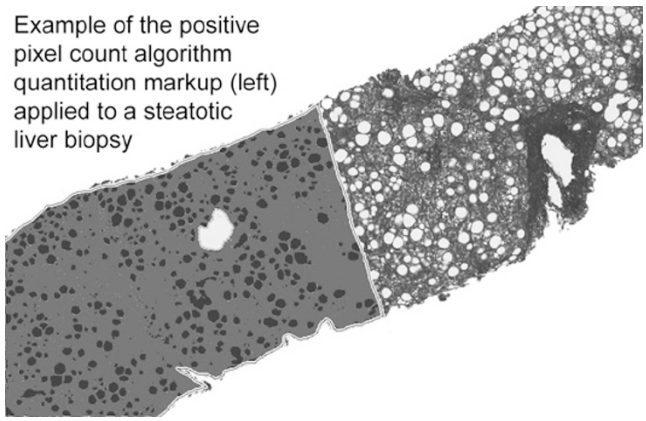
Design: We compared two computerized techniques and contrasted these with radiology measurements (by MRI), and visual steatosis-scoring methods by three pathologists on frozen section slides, permanent slides, and whole slide images (WSIs). The two computerized methods applied to WSIs included a commercial positive pixel count algorithm tuned to quantitate white space and a custom algorithm programmed at our institution. The study cohort included pediatric patients (n=10), adult patients (n=13), and transplant biopsies with corresponding frozen section (n=18) and permanent slides (n=18).

Results: Correlations were observed among measurement methods and clinical parameters with the strongest correlations observed among the pediatric cohort. For all patients, there was a statistically significant correlation between pathology, radiology and each image analysis modality ($r = 0.75\text{-}0.97$, $p < 0.0001$). A statistically significant relationship was observed in all patients between each method, body mass index (BMI) and albumin but not with alanine aminotransferase (ALT) or aspartate aminotransferase (AST).

Correlation r values

All Patients	Positive Pixel Count Algorithm	Custom Algorithm	Pathologist Macro	Pathologist Overall	Radiology
Positive Pixel Count Algorithm	1.00				
Custom Algorithm	0.88*	1.00			
Pathologist Macro	0.75*	0.97*	1.00		
Pathologist Overall	0.66*	0.96*	0.77*	1.00	
Radiology	0.89*	0.95*	0.91*	0.93*	1.00
BMI	0.37*	0.47^	0.49^	0.56^	0.54^
ALT	0.03	-0.01	-0.04	-0.04	0.00
AST	-0.07	-0.10	-0.11	-0.09	-0.09
Albumin	0.64^	0.61^	0.56^	0.55^	0.59^
Pediatric Patients	Positive Pixel Count Algorithm	Custom Algorithm	Pathologist Macro	Pathologist Overall	Radiology
Positive Pixel Count Algorithm	1.00				
Custom Algorithm	0.95*	1.00			
Pathologist Macro	0.87^	0.92'	1.00		
Pathologist Overall	0.89'	0.92'	0.98*	1.00	
Radiology	0.84^	0.92'	0.82^	0.86^	1.00
BMI	0.58	0.59	0.58	0.67^	0.67^
ALT	0.77^	0.83^	0.69^	0.67^	0.78^
AST	0.64	0.65	0.61	0.62	0.44
Albumin	0.07	-0.01	-0.25	-0.26	-0.17

For the correlation: *P < 0.0001, 'P < 0.001, ^P < 0.05
P > 0.05 for all others



Conclusions: The assessment of hepatic steatosis is susceptible to intra- and inter-observer variability among pathologists. Our study demonstrates the utility of image analysis in providing an objective measurement of hepatic steatosis with correlations between qualitative image analysis, pathologist assessments, radiology measurements and several clinical parameters.

1744 CD10 (MME) Upregulation Is Commonly Associated with Hepatocellular Carcinoma Related to Hepatitis C Virus Infection and Chemoembolization

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Background: Downregulation of CD10, also known as membrane metalloendopeptidase (MME), has been shown to be associated with liver metastasis and accelerated tumor growth in colorectal cancer. However, CD10 dysregulation may differ in subsets of hepatocellular carcinoma (HCC) due to the genetic heterogeneity related to variable etiologies and treatments of HCC. This study investigates CD10 expression in HCC and assesses whether rates of expression are affected by viral etiology (HBV and HCV) or chemoembolization (CE).

Design: A total of 86 liver explants and resection specimens for HCC were collected from NYU Langone Medical Center and Bellevue Hospital Center. The cases were categorized based on the type of associated viral infection (HBV vs. HCV), presurgical treatment (with or without CE), tumor focality, size, differentiation, the presence or absence of necrosis, vascular invasion, and patient gender and age. Neoplastic and paired non-neoplastic liver tissue was microdissected and total RNA was extracted from formalin-fixed, paraffin-embedded tissue. The rate of expression of CD10 was measured by quantitative RT-PCR using primers for the CD10 gene. Fischer's exact test was used for statistical analysis.

Results: Downregulation of CD10 expression was observed in 63% (54/86) of HCC cases, and upregulation was seen in only 24% (17/86) of HCC cases. In HCCs without presurgical treatment, there was a high percentage of downregulation (87.2%; 34/39) and a low percentage of upregulation (12.8%; 5/39). However, after CE, there was a significant decrease in the percentage of cases with downregulation (62.5%; 20/32) and an increase in those with upregulation (37.5%; 12/32) ($p<0.05$). Independently, compared to HBV-related HCCs, HCV-related HCCs had a significantly lower percentage of downregulation (57.1%; 12/21 vs. 85.1%; 23/27, respectively) and a higher percentage of upregulation (42.9%; 9/21 vs. 14.8%; 4/27, respectively) ($p<0.05$). No statistical difference was observed in CD10 expression when compared by tumor focality, tumor size, differentiation, necrosis, vascular invasion, or in gender and age.

Conclusions: Chemoembolization appears to upregulate CD10 expression in a subset of HCCs. HCV-related HCCs are less likely to have downregulation of CD10 as opposed to those related to HBV. The findings suggest that chemoembolization may change HCC's biologic behavior by upregulation of CD10 and also demonstrate a molecular difference between HCV and HBV-related HCCs.

1745 Concurrent Increase in Hepatic Mitosis and Apoptosis: A Marker for Hepatic Artery Thrombosis in Transplant Liver Biopsies

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Background: Liver biopsies are critical in managing patients after liver transplantation. One of the key histological patterns in transplant pathology is spotty hepatocyte necrosis without significant lobular inflammation, which is typical of recurrent hepatitis C (HCV). Over the past years, we have noticed a unique pattern of injury that mimicks recurrent HCV, but is not HCV: increased spotty hepatocyte necrosis without significant inflammation, but with increased concurrent hepatocyte mitosis. This pattern was found in individuals transplanted for HCV as well as non-HCV disease. To better understand this unique pattern of injury, we examined the clinicopathological findings, using a control group with typical recurrent HCV.

Design: Cases with this unique pattern of injury and controls of recurrent HCV were identified by computer search of the pathology files. Cases were scored for portal and lobular inflammation (Ishak score). Hepatocyte apoptosis and mitosis were quantified by counting 10 high power fields (HPF).

Results: We identified 8 biopsies with the injury pattern of lobular spotty necrosis and increased mitotic figures, but without significant lobular inflammation. The underlying native liver diseases were HCV (n=5), autoimmune (n=2), and polycystic (n=1). There was an average of 62 days between transplantation and biopsy (range, 8 - 150).

Interestingly, examination of the medical records for these cases found a very strong association with acute hepatic arterial problems: 6/8 cases had acute hepatic artery thrombosis, 1/8 had acute cellular rejection with arteritis, and 1/8 had artery flow abnormalities on ultrasound suggestive of stenosis. In contrast, none in the control group of recurrent HCV had hepatic artery problems ($p < 0.0001$). The HCV control group did not differ from the study group in gender distribution, average age at biopsy, or average time interval between transplantation and biopsy. There was also no significant difference between the two groups in portal or lobular inflammation. In contrast, there was more hepatic apoptosis in the study cases than the controls (average of 10.25 vs. 2.77 apoptotic bodies/10HPF; $p = 0.0004$). Likewise, there were more mitoses in the study cases than the controls (average 6.25 vs. 0.14/10HPF; $p < 0.0001$).

Conclusions: The pattern of increased hepatocyte apoptosis and mitosis and in transplant liver biopsies without significant inflammation is strongly associated with hepatic arterial problems. This previously undescribed histological pattern should prompt evaluation of the hepatic artery.

1746 Primary Liver Carcinoma with Biphenotypic Differentiation: 22 Cases of Combined Hepatocellular Carcinoma-Cholangiocarcinoma

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Background: Combined hepatocellular carcinoma-cholangiocarcinoma (HCC-CCa) is an uncommon tumor accounting for 1 to 5% of primary liver malignancies. By definition, the diagnosis includes tumors with unequivocal elements of hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCa). The 2010 WHO recognized subtypes including the classic type and those with stem-cell features. Several studies show survival intermediate between HCC and CCa. Reported one and three year survival rates are 92% and 77% for HCC, 82% and 47% for HCC-CCa and 44% and 18% for CCa.

Design: Potential cases of biphenotypic primary liver cancer (HCC-CCa) were identified in our Pathology database from 1994 to present. Re-reviewed cases were included only if they showed morphologic and immunohistochemical (IHC) evidence of biphenotypic (HCC-CCa) differentiation. 22 cases met inclusion criteria. IHC included the following antibodies: EpCAM, NCAM, K7, K19 and polyclonal CEA. Staining properties were scored for intensity and proportion of reactive cells. Demographic and retrospective outcome data were collected from medical records.

Results: Cases had a mean follow-up of 25 months; the median overall survival (OS) was 1.3 years. One and three year survival rates were 56% and 33%. 23% of cases occurred in patients with cirrhosis; the remainder did not have underlying liver disease. Average AFP at presentation was modestly elevated (mean 30 ± 53 ng/mL). All tumors showed canalicular staining for pCEA. All tumors expressed EpCAM, K7 and K19 either diffusely or in a mosaic pattern. Significant NCAM expression was seen in only 3 tumors. Poor histopathologic prognostic features were cholangiolar differentiation (median OS 11.3 versus 25.7 months) and diffuse, strong reactivity for EpCAM, K7 and K19 (median OS 10.9 versus 27.4 months).

Conclusions: Primary liver cancer with biphenotypic differentiation (HCC-CCa) is an entity of growing recognition that has different prognostic implications than either HCC or CCa. Similar to prior studies, our study showed a prognosis that was intermediate between those reported for HCC and CCa. Strong expression of EpCAM, K7 and K19 as well as cholangiolar differentiation were associated with a shorter median OS. Without an IHC panel, hepatocellular or cholangiolar differentiation may be under-appreciated in primary liver carcinomas. Additional studies are needed to further examine these findings.

1747 Characterization of Translocations in Mesenchymal Hamartoma of the Liver by Targeted Next Generation Sequencing of Formalin-Fixed Paraffin-Embedded Tissue

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Background: Mesenchymal hamartoma of the liver (MHL) is a rare primary tumor that occurs in the pediatric population, and is in some cases associated with subsequent development of undifferentiated embryonal sarcoma. Prior case studies have demonstrated a recurring t(11;19) translocation, calling into question the lesion's designation as a hamartoma. The translocation breakpoints on chromosomes 11 and 19 have been defined on the basis of cytogenetic studies, and have recently been shown to involve the *MALAT1* gene on chromosome 11 and a gene poor region on termed *MHLB1* on chromosome 19. Given the lack of detailed knowledge of the breakpoint regions, and the availability of only formalin fixed paraffin embedded (FFPE) tissue from most cases, we used targeted next generation sequencing (NGS) from archival FFPE tissue to provide more detail on the genetic events of MHL tumorigenesis.

Design: Sequencing libraries were prepared from 1 ug of genomic DNA extracted from 11 cases of MHL. Target enrichment was then performed using custom cDNA probes targeting *MALAT1* plus 10Kb of flanking sequence and the 22Kb *MHLB1* locus (71Kb total). The captured DNA was then sequenced on a single NGS lane using 2x101bp paired-end reads. Sequence data were aligned and translocation breakpoints identified using the Breakdancer software package.

Results: Sequencing data included 114,568,422 reads, 10% of which mapped to the target regions, resulting in 2,800x coverage. Reads that aligned to the capture regions were analyzed; those in which only one of the ends mapped to a target region were examined as potential translocation breakpoints. We identified putative translocations involving *MALAT1* and regions of chromosomes 3, 4, 8, 16 and 19 in areas of Alu repeats and long interspersed elements. As a validation of the approach, Sanger sequencing was used to confirm the candidate translocation identified in a case known to harbor a t(11;19), and demonstrated a breakpoint involving *MALAT1* and a region of chromosome 19 10 kb centromeric to the *MHLB1* locus.

Conclusions: NGS can be used to characterize translocation breakpoints with single base pair resolution. In the case of MHL, the involvement of highly repetitive regions may indicate that homologous unequal recombination of similar but non-allelic sequences underlies the translocation event. The recurring involvement of *MALAT1* (which encodes a non-translated RNA thought to be involved in the spliceosome) suggests a novel pathway of tumorigenesis due to aberrations in mRNA splicing.

1748 Aldo-Ketoreductase Family 1 B10 (AKR1B10) Protein as a Unique Malignant Biomarker To Distinguish Benign Liver Lesions from Hepatocellular Carcinoma

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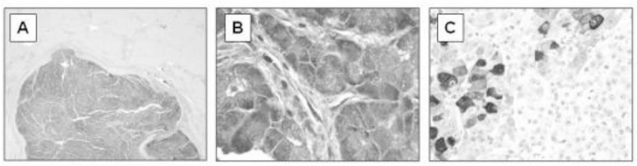
Background: AKR1B10 contributes to detoxification of xenobiotics by lipid peroxidation and metabolizes physiological substrates such as farnesal, retinal and carbonyls. Metabolizing these lipid substrates plays a crucial role in promoting carcinogenesis. AKR1B10 was first isolated from hepatocellular carcinoma (HCC) and further identified to be over-expressed in many cancers from various organs. In the present study, we performed an immunohistochemical (IHC) analysis to determine the prevalence/pattern of AKR1B10 expression in HCC, its usefulness to differentiate benign liver lesions from HCC, and to correlate with etiology and tumor response rates in order to identify a reliable biomarker to predict malignancy and chemosensitivity.

Design: Explanted livers with HCC with or without chemoembolization therapy (n=58) were retrieved from an achieved pathology database. Pathology reports and slides were reviewed to establish tumor response rate and correlated with AKR1B10 expression and therapy regimen. The etiologies of HCC included Hepatitis B and C, autoimmune hepatitis, steatohepatitis, alcohol, and cryptogenic cirrhosis. Benign hepatic lesions including focal nodular hyperplasia (FNH) (n=8) and hepatic adenoma (n=21), as well as HCC arising in an adenoma (n=3) were also included. IHC was performed using a monoclonal mouse AKR1B10 antibody and the avidin-biotin-peroxidase approach. Positive staining was defined as granular, cytoplasmic staining. The staining was further classified as no staining (0-10%), minimal (10-50%), moderate (50-75%) and high (>75%).

Results: Strong expression of AKR1B10 was observed in 87.9% (51/58) of HCC, but not adjacent normal/cirrhotic tissue (see Panel A). Greater than 75.8% of tumors (44/58) demonstrated high levels of expression (see Panel B). There was no correlation between the etiology of HCC or tumor response rates/therapeutic regimen with the extent of AKR1B10 expression. No expression of AKR1B10 was detected in cases of adenoma or FNH. In contrast, AKR1B10 was expressed in 66.7% (2/3) of HCC arising in the setting of adenoma, but only in the malignant component (see Panel C).



Conclusions: AKR1B10 expression levels in HCC are high irrespective of tumor etiology and tumor response rates. This finding likely explains why HCC is often unresponsive to chemotherapeutic/interventional approaches. Moreover, AKR1B10 expression can be used to reliably differentiate between benign and malignant primary lesions of the liver.

1749 Non-Alcoholic Steatohepatitis (NASH) Influences the Disease Progression of Chronic Hepatitis C

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Background: Non-alcoholic steatohepatitis (NASH) is a chronic liver injury with liver histology of steatosis, lobular inflammation and fibrosis (pericellular and perivenular) in nonalcoholic patients. The occasional occurrence of NASH in chronic hepatitis C (CH-C) has been reported, but the influences of NASH on the disease progression of CH-C are obscure. Therefore, in the present study, we examine the influences of NASH on the disease progression of CH-C.

Design: Liver biopsies from 400 cases with CH-C were examined histologically. The histology of CH was evaluated by the activity scores (A0, A1, A2, and A3) and fibrosis scores (F0, F1, F2, F3, and F4 = cirrhosis). NASH was evaluated by the grading (G1, G2, G3, and G4) and staging system (S1, S2, S3, and S4) according to the Brunt system (Brunt EM, et al. Am J Gastroenterol 94:2467-2474, 1999).

Results: A morphological pattern of NASH was present in 69 cases (69/400, 17%). The incidence of NASH did not correlate to the degree of activity and fibrosis of CH-C. In the 69 cases with NASH, the grade of NASH and the activity of CH-C did not correlate, but the stage of NASH and the fibrosis of CH-C significantly correlated ($P < 0.001$). In 18 cases (10 with F3, A2, and 8 with F3, A3) with NASH, various fibrosis patterns (portal, periportal, septal, pericellular and perivenular) causing both CH-C and NASH were admixed.

Incidence of NASH in each group of activity and fibrosis system of CH-C

	F0	F1	F2	F3	F4
A0	6/37 (16%)	5/17 (29%)	0/1 (0%)	0/1 (0%)	0 (0%)
A1	5/71 (7%)	18/111 (16%)	4/18 (22%)	0/10 (0%)	0/11 (0%)
A2	0/5 (0%)	0/14 (0%)	10/35 (28%)	10/27 (37%)	3/20 (15%)
A3	0/1 (0%)	0/2 (0%)	0/2 (0%)	8/11 (72%)	0/6 (0%)

Conclusions: In 18 cases (18/400, 4.5%), pericellular and perivenular fibrosis caused by NASH played a role on the destruction of hepatic lobular structure and the disease progression. In such cases, these changes lead to cirrhosis, so the treatment of NASH is necessary to prevent the disease progression of CH-C.

1750 Immunophenotypic Subtypes of Hepatic Adenomas in a Large Tertiary Care Center in the United States

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Background: Hepatic adenomas (HAs) are benign tumors with the capacity for malignant transformation. A classification scheme for HAs was recently proposed by Bioulac-Sage et. al. Three subtypes of HAs were identified based on molecular and immunophenotypic characteristics: inflammatory type (IHA) with Serum Amyloid A overexpression, steatotic type correlated with hepatic nuclear factor (HNF) *1A* mutation (SHA), and beta-catenin-mutated subtype with upregulation of glutamine synthetase (bHA). The bHA subtype has a higher risk of transformation into hepatocellular carcinoma, and so detection of this subtype provides important prognostic information.

Design: We identified 30 cases of hepatic adenomas in our archives from 1994 to 2011, sampled from 27 patients. Three patients had both biopsies and resections, eight patients had only biopsies, and 16 had only resections. Immunohistochemistry (IHC) was performed on paraffin sections with the following 3 antibodies: anti-liver fatty acid binding protein (LFABP), anti-glutamine synthetase (GS) and anti-Serum amyloid A (SAA). Subtype classification was analyzed based on H&E morphology. The staining of IHC was scored from 0-2, with 0 being 0-9% staining, 1 being 10-50% staining in the neoplastic cells, and 2 being >50% in neoplastic cells. Immunosubtyping of HAs based on IHC results was performed and compared to the morphological subtypes.

Results: There were 21 females (ages 6-75) and 6 males (ages 24-66) in our cohort. Eleven patients had multiple adenomas. The adenomas averaged 8.7cm in greatest dimension. On morphology, 11 were classified as bHAs, 9 as IHAs, and 10 as SHAs. For patients with multiple adenomas, 3 showed bHA, 3 IHA, and 5 SHA phenotypes. All HAs were positive for at least one IHC marker. GS was positive in 9 out of 11 bHAs (81%), it was also seen in 4 out of 9 IHAs. SAA staining was positive in 8 out of 9 (89%) IHAs, but some degree of staining was also observed in 7 of 11 (64%) of bHA, with 2 having focal and 5 diffuse staining. LFABP expression was lost in 8 of 10 (80%) SHA subtype.

Conclusions: LFABP, GS and SAA were highly sensitive immunomarkers for diagnosing SHA, bHA and IHA subtypes, respectively, and may be useful in confirming morphologic diagnosis. Furthermore, 64% bHA stained positively for SAA and the majority of these lesions had atypical features (4 out of 5 cases). The behavior of these "overlap" lesions should be studied further, as they have characteristics of both bHA and IHA. Molecular analysis may help further define these cases with both GS and SAA overexpression.

1751 Clathrin Heavy Chain Expression in Atypical Hepatic Adenomas

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Background: Hepatic adenomas (HAs) are benign neoplasms with the potential to undergo malignant transformation. One of 3 immunophenotypically characterized subtypes of HAs, beta-catenin mutated HAs with glutamine synthetase (GS) overexpression, is considered the most likely to become malignant. The criteria for histologic atypia in HAs and their corresponding immunohistochemical (IHC) profiles to define malignant potential are not well established. Clathrin heavy chain (CHC) has recently been established as a marker for the diagnosis of hepatocellular carcinoma, but its staining profile in HAs has not yet been investigated. In this study of atypical HAs, we sought to establish immunophenotypical criteria for atypical HAs, including the new marker CHC.

Design: We searched our database for all HAs from 1994 to 2011 and identified 5 out of 27 cases of HAs with atypical features by light microscopy. All cases were free of disease in the surrounding liver. Architectural atypia was defined as the presence of trabecular or pseudoacinar formation. Cytologic atypia was defined as nuclear pleomorphism, prominent nucleoli, and increased mitotic activity. Reticulin special stain and immunostains for CD34, GS and CHC were performed.

Results: Atypical HAs were seen in five patients including 2 females (ages 7 and 11) and 3 males (ages 22-66). Two patients had multiple adenomas. The HAs averaged 12 cm in greatest dimension. On histology, all five were classified as beta-catenin activated HAs. No steatotic or inflammatory HAs had atypical features morphologically. No stromal invasion was identified in any of the atypical HAs. By IHC analysis, four specimens were positive for GS and one was positive for Serum Amyloid A (SAA). SAA reactivity was also seen in 3 cases which were GS positive. All five cases were positive for CHC with >40% staining in the neoplastic cells. Interestingly, the CHC staining was stronger in areas of increased cytologic and architectural atypia. Focal loss of reticulin staining and increased vascularization by CD34 was seen in three of five cases.

Conclusions: This preliminary study identifies 5 out of 27 (19%) HAs show morphologically atypia and majority of the atypical HAs have GS overexpression and likely to be beta-catenin activated. All atypical HAs express CHC and the expression appears to correlate with the degree of cytologic or architectural atypia. Further analysis of CHC expression in other types of HAs is underway. Clincal follow up information and larger prospective study is needed to help indentify the risk of malignant transformation in patients with atypical HAs.

1752 Pediatric Fulminant Hepatic Failure of Unknown Etiology – A Unique Immune Mediated Mechanism of CD8-Positive T-Cell Activation Causing Simultaneous Bone Marrow Suppression

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Background: In the vast majority of pediatric fulminant hepatic failure (FHF), the etiology of liver disease was unknown despite a series of studies for viral hepatitis, toxic or metabolic liver disease. The aim of this study is to characterize clinical and pathological features of FHF of unknown etiology (indeterminate FHF) in pediatric liver transplant population and to investigate a possible pathogenesis.

Design: From 2005 to 2011, 28 out of 161 pediatric liver transplants in National Center for Child Health and Development were FHF. Among FHF, 24 were of indeterminate etiology. The causes of other four recipients with FHF were viral infections (CMV, EBV, echo virus) and mitochondrial hepatopathy. The clinical information was obtained from medical record. Pathological analysis was performed using explanted liver and allograft. For immunohistochemistry, antibodies for anti-cCD3, CD4, CD8, CD20, CD56, perforin, TIA-1, FOXP3, and C4d were used.

Results: The median age at liver transplant was 10 month-old (range, 38 day to 148 month). 14 patients out of 24 (58%) were under 12 month-old. All patients showed pancytopenia and bone marrow examination revealed hypocellular marrow and macrophage activation with secondary hemophagocytosis. Explanted liver showed massive necrosis with lymphohistiocytic infiltration. CD8+T-cells were dominant population and infiltrated not only portal area but sinusoid. Eight patients (33%) including 22 biopsies demonstrated acute cellular rejection(ACR) with classic portal features. Seven patients (29%) including 34 biopsies demonstrated centrilobular injury. No ACR or centrilobular injury was seen in recipients with FHF of known causes. Idiopathic lobular hepatitis was seen in 10 recipients. Aplastic anemia (AA) developed in two recipients. A skewing pattern of HLA was observed.

Conclusions: Pediatric indeterminate FHF frequently occurred in young infant and presented massive necrosis with a striking CD8-positive T-cell accumulation in liver, bone marrow suppression, and refractory rejection with centrilobular injury. High incidence of AA in children with FHF and CD8+T-cell increase in peripheral blood of AA associated FHF were reported. Those observations strongly support the hypothesis that immune-mediated mechanism by activated CD8+T-cells is involved in the etiology of pediatric indeterminate FHF.

1753 Utility of HSP70, Glutamine Synthethase and Glypican-3 in Distinguishing Hepatocellular Adenoma from Well-Differentiated Hepatocellular Carcinoma

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Background: Well-differentiated hepatocellular carcinoma (WD-HCC) can mimic high-grade dysplastic nodule (HGDN) in cirrhotic liver and hepatocellular adenoma (HA) in non-cirrhotic liver. Combined use of heat shock protein 70 (HSP70), glutamine synthetase (GS) and glypican-3 (GPC) has been shown to help in distinguishing HGDN from HCC. This study evaluates the efficacy of this panel in distinguishing HA from WD-HCC.

Design: Immunohistochemistry for HSP70, GS and GPC was done in typical HA (n=24), HGDN (n=16), adenoma-like HCC (n=7) and WD-HCC (n=41;19 non-cirrhotic, 22 cirrhotic). Adenoma-like HCCs were tumors that resembled HA, but in which a diagnosis of HCC was based on focal atypical morphological features like small cell change, cytologic atypia, prominent pseudoacini and/or reticulin loss. The staining intensity was graded from 0-3. GS was considered positive if 2+ or 3+ staining was observed in ≥50% of tumor cells. GPC and HSP70 were scored positive if 2+ or 3+ staining was seen in ≥25% of tumor cells.

Results: Expression of HSP70, GS and GPC was seen more commonly in WD-HCCs than typical HAs and DNs. Expression in WD-HCC and adenoma-like HCC was similar. The overall sensitivity was highest with HSP70, which stained nearly two-thirds of WD-HCC, while GS and GPC were positive in one-third of cases. Positivity for ≥2 markers was seen in 17(41%) WD-HCCs and 3(43%) adenoma-like HCCs; this was rare in DN (1/16) and not seen in typical HA.

	HSP70	GPC	GS	HSP70+GPC	HSP70+GS	GPC+GS	HSP70+ GPC+GS
HA, typical (n=24)	3 (13%)	0 (0%)	1 (7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Adenoma-like HCC (n=7)	4 (57%)	1 (14%)	3 (43%)	1 (14%)	3 (43%)	1 (14%)	1 (14%)
DN (n=16)	3 (19%)	1 (6%)	2 (13%)	1 (6%)	1 (6%)	1 (6%)	1 (6%)
WD-HCC (n=41)	25 (61%)	13 (32%)	15 (37%)	11 (27%)	11 (27%)	7 (17%)	6 (15%)
p value*	0.007	0.001	0.002	0.004	0.004	0.032	0.054

*typical HA vs. WD-HCC

Conclusions: HSP70, GS and GPC are expressed significantly more often in WD-HCC than typical HA and HGDN, but the overall sensitivity of these markers is not high. Positivity for ≥2 of these markers was not observed in typical HA and strongly supports HCC, although less than half of WD-HCC were positive for ≥2 markers. The staining pattern of adenoma-like HCC closely resembled WD-HCC. Since these tumors are most likely to be confused with typical HA, this combination of stains is likely to be useful in clinical practice.

1754 The Central Zone "Portalization" Phenomenon in Nonalcoholic Steatohepatitis (NASH)

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Background: Ferrell and collegues (AASLD abstract, 2007) have reported the presence of arterialized vessels and ductular structures within fibrosed central zones (CZ) in cases of NASH. We sought to further characterize this phenomenon with the aid of glutamine synthetase (GS) – a marker of CZ hepatocytes.

Design: 46 liver biopsies from patients with NASH (M=19, F=27, mean age 48.1 [range 9-53]), stages 1a-3 were evaluated by H&E, trichrome and double GS/CK-7 and GS/SMA IHC. Special attention was given to CZ findings. Nine biopsies showing simple steatosis were used as controls.

Results: Our study has confirmed the frequent occurrence of arterioles and ductular structures in fibrotic CZs in NASH. The concomitant presence of "dense" fibrosis and inflammation may impart a distinct "portal tract-like" appearance to these CZs, which may be confused with true portal tracts on routine stains. Double staining using either

CK7 or SMA with GS greatly facilitated the recognition of portalized central areas and allowed the recognition of central-portal approximation and/or fusion in most cases, indicating that parenchymal extinction and subsequent linking of portal and central spaces with ingrowth of portal structures into CZs likely represents the underlying mechanism of centrilobular "portalization" in NASH. Interestingly, migration of putative hepatic progenitor cells (HPC) (very small, strongly CK7+ cells within the hepatic lobule) into CZs was identified in all cases showing centrilobular arterioles or ductules and in some cases with earlier CZ changes. Associated features are shown in Table 1. Simple steatosis controls showed none of the above CZ abnormalities.

Table 1. Histologic Features Associated with CZ "Portalization"

	CZ arteries or ductules present (n=24)	CZ arteries or ductules absent (n=22)	P value
NAS* (median, range)	4 (3-7)	5 (3-7)	NS
NAS ballooning = 2	10 (41.6%)	2 (9%)	0.01
Mallory bodies	22 (91.6%)	14 (63.6%)	0.02
Stage >1a (Kleiner & Brunt)	21 (87.5%)	13 (59%)	0.02
Ductular reaction	11 (45.8%)	3 (13.6%)	0.01
Dense CZ fibrosis	17 (70.8%)	6 (27.2%)	0.003
CZ chronic inflammation	16 (66.6%)	4 (18.1%)	0.0009
Putative HPCs in CZs	24 (100%)	12 (54.5%)	0.0002

*NAS = NAFLD Activity Score

Conclusions: The phenomenon of CZ "portalization" in NASH is common and occurs due to parenchymal extinction and portal-central approximation/fusion. This phenomenon represents a potential diagnostic pitfall. The identification of HPCs extending into "portalized" CZs represents a novel observation that may be related to the pathogenesis of the centrilobular lesion in NASH and merits further investigation.

1755 Immunohistochemistry (IHC) for c-Myc in the Differential Diagnosis of Vascular Tumors of the Liver

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Background: Angiosarcoma (AS) is a rare, aggressive malignancy that may involve the liver, either primarily or as a metastasis. The distinction of hepatic AS from potential morphological mimics, including low-grade malignant vascular tumors such as epithelioid hemangioendothelioma (EHE) and benign lesions including hemangioma (HEM) and peliosis hepatis (PH), is clinically significant and may be difficult in some cases. For this reason there has been continued interest in the development of ancillary markers that may assist in the differential diagnosis of vascular tumors. Recently, up-regulation of *c-Myc* has been reported to be a common event in AS, and IHC for c-Myc has been shown to be helpful in evaluating post-radiation vascular lesions of the breast. We evaluated c-Myc IHC in a series of well-characterized hepatic vascular tumors.

Design: Formalin-fixed, paraffin-embedded blocks from 13 AS, 8 EHE (including 3 with high-grade cytology), 14 HEM and 2 PH were immunostained for c-Myc using a commercially available antibody and the Dako Envision detection system. Cases were scored as "positive" and "negative" based on the presence of at least focal nuclear immunoreactivity for c-Myc in endothelial cells.

Results: Nuclear c-Myc expression was seen in 10/13 (77%) AS, significantly higher than in EHE 0/8 (0%) (p=0.001) and HEM, 2/14 (14%) (p=0.002). The two HEM positive cases were both cavernous type. 2 cases of PH were also negative. The sensitivity and specificity of c-Myc expression for the diagnosis of AS were 77% and 95%, respectively. c-Myc expression was also occasionally noted in hepatocytes, a potential pitfall.

Conclusions: IHC for c-Myc shows high sensitivity and specificity in the differential diagnosis of AS involving the liver from potential mimics, both malignant and benign. Absent c-Myc expression in EHE, including cases showing high-grade cytology, is diagnostically useful and suggests that the molecular pathogenesis of EHE differs from that of conventional AS. On-going FISH studies for c-Myc amplification should help to clarify the significance of limited c-Myc expression in cavernous HEM, a lesion which is unlikely to be mistaken for AS in most instances.

1756 The Effect of the Etiology of Cirrhosis on Glypican-3 Expression in Hepatocellular Carcinoma

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Background: Overexpression of Glypican-3 (GPC3), a cell membrane bound proteoglycan which modulates hepatocyte growth and differentiation, occurs frequently in hepatocellular carcinoma (HCC); an immunhistochemical stain for GPC3 is a useful adjunct in the diagnosis of HCC. Most HCCs occur in cirrhotic livers, but how the etiology of cirrhosis affects GPC3 expression in HCC has not been studied in detail. Because chronic hepatitis C (HCV) and steatohepatitis are the most common causes of cirrhosis, we aim to compare GPC3 expression between HCC arising in cirrhosis due to chronic hepatitis C (HCV) and HCC arising in cirrhosis due to steatohepatitis.

Design: The immunohistochemical expression of GPC3 in 60 patients with HCC occurring in explanted livers with HCV (n=30) and steatohepatitis (30; 10 alcoholic, and 20 non-alcoholic) related cirrhosis was examined. HCC was graded using WHO classification criteria (G1: well differentiated, G2: moderately differentiated, and G3: poorly differentiated). All tumors were scored for cytoplasmic and membranous staining. A tumor was considered positive for GPC3 if >10% of the neoplastic cells showed cytoplasmic and membranous immunoreactivity. The expression of GPC3 in HCC was correlated to clinicopathologic features, including etiology of cirrhosis, tumor differentiation, and focality of tumor.

Results: There was no difference of tumor differentiation between HCV associated HCC (6 G1, 18 G2, and 6 G3) and steatohepatitis associated HCC (5 G1, 17 G2 and 8 G3), p= 0.82. GPC3 expression occurred significantly more frequently in HCV associated HCC (83%, 25 of 30) compared to steatohepatitis associated HCC (50%, 15 of 30), p= 0.01. In HCV related HCC, 83% of G1, G2 and G3 tumors were positive for GPC3. In steatohepatitis related HCC, 60% of G1, 36% of G2, and 63% of G3 tumors were positive for GPC3. In both groups, GPC3 expression showed no correlation with tumor differentiation. Etiology of steatohepatitis (alcoholic vs non alcoholic) did not correlate with GPC3 expression, p= 0.14. Multifocal HCC occurred in 66 % (20 of 30) of HCC associated with HCV and in 50% (15 of 30) of HCC associated with steatohepatitis and did not correlate with GPC3 expression, p= 0.055.

Conclusions: HCC arising in cirrhosis due to HCV is more likely to express GPC3 than HCC arising in cirrhosis due to steatohepatitis. Hence, the significance of GPC3 immunostain results in hepatic tumors should be interpreted in light of the patient's underlying liver disease. The etiology of this difference is unknown but GPC3 activation may have a role in HCV induced carcinogenesis.

1757 Interleukin-28B Polymorphisms Are Associated with Hepatic Fibrosis and Inflammatory Molecular Changes Following Liver Transplantation in Patients with Hepatitis C Infection

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Background: Chronic hepatitis C is the leading indication for liver transplantation. Recurrence of hepatitis C after transplantation is universal and response rates to standard antiviral therapy are poor. Recently, genetic variation in the interleukin-28B (IL28B) gene, encoding interferon lambda3, has been associated with viral clearance in nontransplant and transplant patients. Histological and molecular consequences of this genetic variation are unknown. We aimed to gain further insight into effects of IL28B variants on hepatic tissue.

Design: We studied histopathology and large-scale gene expression by Affymetrix genechip in 25 liver transplant biopsies from 13 recipients with recurrent hepatitis C. Donor and recipient DNA were typed for IL28B gene polymorphisms *rs12979860 and rs8099917* using ABI TagMan allelic discrimination kit. We related IL28B genotypes with histological activity, fibrosis stages, and gene expression profiles. We also related the transcripts to histopathology and clinical parameters.

Results: The frequencies of IL28B donor genotypes at rs12979860 were 5 C/C, 6 C/T, and 2 T/T and at rs8099917 8 T/T, 5 T/G, and 0 G/G. *IL28B recipient genotyping is underway.* The donor rs12979860 C/C genotype was related with higher histological hepatic fibrosis stages, portal inflammation, bile duct injury, and venulitis when compared to C/T and T/T genotypes. The donor rs8099917 T/T genotype was related with high fibrosis stages. Furthermore, rs12979860 C/C genotype was associated with increased intrahepatic expression of T cell and plasma cell (immunoglobulin) transcripts and decreased parenchymal transcripts, and rs8099917 T/T genotype is related with increased plasma cell transcripts. HCV viral loads were not different among IL28B genotypes. Ishak hepatitis activity scores strongly correlated with IFNG-dependent transcripts, T cell and macrophage-associated transcripts, and decreased liver transcripts. Indices of hepatocellular injury (ALT, AST, total bilirubin) were strongly correlated with increased IFNG-dependent transcripts and macrophage transcripts.

Conclusions: *IL28B rs12979860 C/C and rs8099917 T/T genotypes are associated with more severe histological recurrence of hepatitis virus C infection with plasma cell rich infiltration.* The molecular burden in liver biopsies reflects hepatitis C activity and fibrosis stage.

1758 Poorly Cohesive Cell (Diffuse-Infiltrative/Signet-Ring) Carcinomas of the Gallbladder (GB): Clinicopathologic Analysis of 24 Cases Identified in 628 GB Carcinomas

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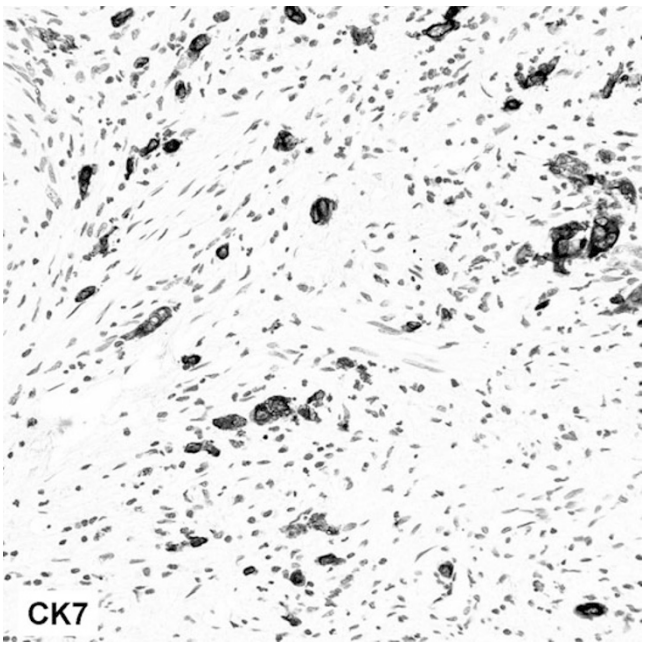
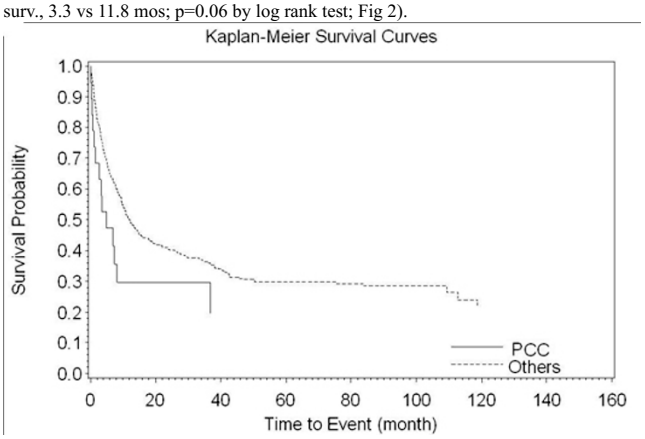
Background: There is virtually no data on the gallbladder counterparts of carcinomas with single-cell or cord-like infiltration, previously called "diffuse-infiltrative" type or "signet-ring cell" (SRC), and now designated as "poorly cohesive cell" (PCC) type in the WHO-2010 classification(Fig 1).



Design: 628 invasive GB CA were reviewed. 24 cases in which classical PCC/SRC pattern constituted > 50% of the tumor were subjected to detailed analysis and contrasted with ordinary GBCs. Excluded were 18 cases in which PCC pattern was focal (<50%), and 8 with dyshesive SRCs floating within the mucin but not infiltrating into the stroma.
Results: F/M=19/3=6.3. Mean age=63. Gross and low-power findings were highly akin to "linitis plastica". Cytologic features varied from small bland-monotonous cells resembling that of "invasive lobular breast ca", to high-grade with pleomorphic nuclei, with coarse chromatin and prominent nucleoli, akin to those of "urothelial plasmacytoid" ca. All cases showed at least focal signet-ring morphology and this was predominant in 50%. Most cases had advanced carcinoma (pT3+, 79% vs 51%; p<0.01). 4/6 cases with LNs available showed mets (vs 38%; p=0.3). While perineural invasion was high (74 vs 38%; p<0.01), vascular invasion was present only in 8 cases (33 vs 72%; p<0.01). The clinical course appeared to be even more aggressive than ordinary GBC (median surv., 3.3 vs 11.8 mos; p=0.06 by log rank test; Fig 2).



Conclusions: Gallbladder counterpart of the carcinoma type that is now classified as "poorly cohesive cell" by the WHO-2010 (previously called "diffuse-infiltrative/signet-ring cell") occurs in 3.8 % of cases with GBC. It is seen almost exclusively in women and appears to have a more aggressive clinical course than ordinary GBC.

1759 BRUCE: A Novel Protein Associated with Carcinogenesis of Liver and Potential Marker for Differential Diagnosis

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Background: BRUCE is a recently found protein with multifunction. One of its functions is to regulate cell division, and reduction in the levels of BRUCE protein by gene silencing results in aneuploid cells. However, it is unknown whether such reduction associates with human liver tumors and if so, whether it can be used as a differential diagnostic and prognostic marker for patients with liver lesions.
Design: Thirty two resections or biopsy cases of human liver mass lesions were studied. The specimens include 18 HCC, 9 adenomas and 5 focal nodular hyperplasia (FNH). Ten HCC cases have cirrhotic background and the remaining 8 do not. Immunohistochemistry was performed using an anti-BRUCE monoclonal antibody. The percentage and intensity (0-3) of the positivity in both lesional and non-lesional hepatocytes were evaluated and scored.
Results: BRUCE protein is detected in the cytoplasm of hepatocytes. All HCCs (100%) show decreased expression of BRUCE at various levels, of which 13/18 (72%) cases have negative stain in more than 50% of tumor cells and the remaining 5 tumors totally lost BRUCE expression. All adenomas and FNHs express BRUCE with 100% of cell positivity for all cases. All cases of hepatocytes adjacent to HCC in either cirrhotic or non-cirrhotic livers show 100% of positivity except 1 cirrhotic liver has 90% of cell stain. The intensity of positive stain varies in non-lesional hepatocyte, FNH, adenoma and HCC; however, the majority (17/23, 74%) of hepatocytes in non-lesional areas have strong (3+) positivity while most (9/13, 69%) of positive HCC show weak (1+) expression and remaining 4 cases are intermediately (2+) positive. 11/14 (79%) of adenoma and FNH have intermediate (2+) or strong (3+) positivity. Loss of BRUCE expression is not associated with the grade and stage of HCC.
Conclusions: The present study, for the first time, demonstrates that loss of BRUCE expression is associated with the development of human HCC. The finding also strongly suggests that BRUCE negative stain may be used as a specific marker to distinguish well-differentiated HCC from benign liver mass lesions, especially for difficult needle biopsy specimens. Further study with a larger number of specimens is needed to confirm our finding, investigate correlation of BRUCE with different etiology, therapeutic response, and survival.

1760 Comparison of Hepatocellular Markers for Diagnosis of Poorly-Differentiated Hepatocellular Carcinoma: High Sensitivity with Combined Use of Arginase-1 and Glypican-3

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Background: Evidence-based use of immunohistochemistry is important in poorly-differentiated liver tumors,especially when limited tissue is available. Commonly used hepatocellular markers like Hep Par 1(Hep) and polyclonal carcinoembryonic antigen(pCEA) have low sensitivity for poorly differentiated HCC(PD-HCC). This study compares the efficacy of four hepatocellular markers in HCC.
Design: Immunohistochemistry for Hep,pCEA, glypican-3(GPC) and arginase-1(Arg) was done on well-(WD),moderately- differentiated(MD) and PD-HCC cases (n=75). In 13 cases,there was >1 differentiation pattern,which were scored separately yielding 88 observations. Staining intensity of 2+ or 3+ (scale 0-3) in >5% of tumor cells was considered positive.
Results: Arg and Hep had the highest sensitivity for WD-HCC,while Arg and GPC had the highest sensitivity for PD-HCC. When staining of >50% of tumor was considered positive,Arg was the most sensitive marker for all differentiations. The addition of Hep or pCEA to Arg did not lead to increased sensitivity. Combined use of Arg and GPC yielded 100% sensitivity for PD-HCC. When staining of >50% of tumor was considered positive,the sensitivity of combined Arg and GPC was 94%.

Table 1

	Arginase-1	Glypican-3	HepPar-1	pCEA
Well-differentiated (n=13)	100/100	61/15	100/100	92/77
Moderately-differentiated (n=40)	100/98	82/60	98/83	88/65
Poorly-differentiated (n=35)	97/80	85/74	63/31	60/17

Numbers stated as a/b reflect percentages; a-positivity in >5% of tumor, b-positivity in ≥ 50% of tumor

Table 2

	Hep+GPC	Hep+Arg	GPC+Arg
Well-differentiated (n=13)	100/100	100/100	100/100
Moderately-differentiated (n=40)	100/98	100/98	100/100
Poorly-differentiated (n=35)	97/85	97/80	100/94

Numbers stated as a/b reflect percentages; a-positivity for at least one marker in >5% of tumor, b-positivity for at least one marker in ≥ 50% of tumor

Conclusions: Arg is the most sensitive marker for all differentiations of HCC. For WD and MD-HCC, addition of other hepatocellular markers to Arg does not significantly improve sensitivity. GPC is of limited value in WD and MD-HCC, but has high sensitivity for PD-HCC. Its combined use with Arg identified all cases of PD-HCC with >50% staining in tumor cells in nearly all cases, indicating that this combination will be helpful in needle biopsies.

1761 Collagen Immunohistochemical Stains in the Liver Are Useful in Differentiating Capsular from Septal Fibrosis

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Background: Cirrhosis is a significant cause of morbidity and mortality with increasing incidence worldwide. While progress has been made in clinical and radiographic diagnosis of cirrhosis, core needle biopsy (CBx) remains essential. It is not unusual to receive severely fragmented CBx, particularly when significant fibrosis is present. The distinction between capsular/subcapsular (Cap) fibrosis and true septal (Sep) fibrosis can be challenging in fragmented CBx, leading to potential over-staging of fibrosis. Collagens and glycoproteins are natural components of hepatic parenchyma, which accumulate in varying proportions as hepatic fibrosis progresses. We evaluated the utility of staining for collagen types and common glycoproteins in differentiating Cap from Sep fibrosis.
Design: Consecutive explanted cirrhotic livers (15) were identified and whole-sections containing adequate Cap and Sep fibrosis were stained for Vitronectin, Orcein, Laminin, Trichrome and Collagens III, IV, V and VI. Staining was graded as negative (0), weak (1) or strong (2). Statistical analysis was performed using a paired T-test. To attempt to mimic the clinical problem of CBx with fragmentation, CBx were obtained from 5 additional explanted cirrhotic livers with inked capsules using an 18-gauge biopsy needle. These were evaluated as previously described.

Results:

Staining Patterns in Cap and Sep Fibrosis in Whole Sections

		Collagen III	Collagen IV	Collagen V	Collagen VI	Vitronectin	Orcein
Sep Fibrosis	strong	14	14	0	15	9	12
	weak	1	1	0	0	4	3
	negative	0	0	15	0	2	0
Cap Fibrosis	strong	6	0	3	7	13	15
	weak	6	10	10	8	1	0
	negative	3	5	2	0	1	0
p-value		0.0012	<0.0001	<0.0001	0.0013	0.2377	0.0824

Collagen III, IV and VI showed statistically decreased staining in Cap compared to Sep fibrosis. Collagen V showed statistically increased staining in Cap compared to Sep fibrosis. Orcein and trichrome were strong regardless of location. Laminin was consistently negative in both regions, but was helpful in identifying the layer of mesothelial cells overlying the capsule. Vitronectin showed the greatest degree of heterogeneity. Analysis of 5 CBx showed that decreased Collagen III, IV and VI and increased Collagen V distinguished Cap from Sep fibrosis with at least 2 of these staining patterns noted in each CBx. Collagens IV and V were the most useful discriminators.

Conclusions: Immunohistochemical staining for collagens III, IV, V and VI show distinct staining patterns in Cap and Sep fibrosis, which can be helpful to distinguish these areas even on small fragmented biopsies.

1762 The p53 Negative Regulator *MDM4* Is Amplified and Over-Expressed in Hepatoblastoma

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Background: The p53 tumor suppressor pathway is inactivated in virtually all cancers. In many tumors, amplification or over-expression of *MDM4* and *MDM2* abolish the p53-mediated oncogenic stress response by inactivating the wild-type p53 protein. Restoring p53 function using inhibitors to disrupt the MDM4/MDM2/p53 interactions is a promising new therapeutic strategy.

Hepatoblastoma (HB) is a highly aggressive neoplasm of childhood. While most HBs are managed with surgery and chemotherapy, no effective treatment exists for refractory and recurrent tumors. Since *TP53* mutations are rare in HB, we posited that a systematic evaluation of *MDM4* and *MDM2* amplification and over-expression in HB may reveal a common mechanism of p53 inactivation and a potential therapeutic target.

Design: Archival specimens (*n*=26) were obtained with IRB approval. In cases with double minutes (dmins) on cytogenetics, spectral karyotyping (SKY) and array comparative genomic hybridization (aCGH) was used to fine-map the minimum genomic interval. FISH analyses with *MDM4* and a chromosome 1q control probe were performed on 26 FFPE samples. 100 cells were counted and scored as amplification (>5 copies), copy-gain (3-4 copies), chromosome 1 polysomy (>2 copies of both probes), or normal. Real-time qPCR for *MDM4, MDM2* and p21 was performed on 21 frozen samples. Data in triplicate was normalized to *GAPDH* and plotted as fold-change compared to normal liver. MDM4 expression was evaluated by immunohistochemistry.

Results: In 2 HB cases with dmins, SKY and aCGH analyses mapped to a ~1 Mb region on Chromosome 1q32.1 containing *MDM4*. *MDM4* FISH analysis identified genomic amplification in 2 cases and copy gain in 8 cases (10/26 cases, 38.4%). Five additional cases had 1-2 extra copies of *MDM4* and chromosome 1 polysomy. *MDM4* expression was 2- to 53-fold up-regulated in 8/21 (38%) cases, including two cases without amplification or copy gain. Nuclear MDM4 protein was detected in amplified cases. *MDM2* expression was however increased in only 1 case. Expression of the p53 transcriptional target, p21, was >2-fold down-regulated in 7/21 (33%) cases, suggesting p53 pathway suppression downstream of MDM4 over-expression.

Conclusions: *MDM4* amplification/copy gain and over-expression, as detected by interphase FISH and qPCR, is a common mechanism by which wild-type p53 can get inactivated in HBs. In contrast, *MDM2* over-expression is a rare event in our series. Our current studies are evaluating the efficacy of small molecule inhibitors in restoring p53 function in the HepG2 hepatoblastoma cell line.

1763 Plasma Cell Hepatitis in Post-Liver Transplant HCV-Infected Patients: The Columbia University Experience

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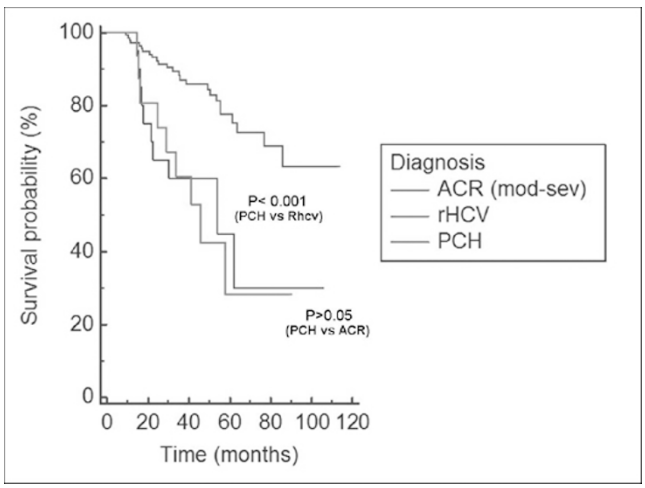
Background: Plasma cell (PC) hepatitis (PCH) in the setting of HCV infection in the post-liver transplantation (LT) period represents a management challenge. Studies showed that PCH likely represents a variant pattern of rejection/immune-mediated graft injury rather than recurrent hepatitis C (rHCV), and may confer poor prognosis. We describe our center's experience with PCH in an attempt to further elucidate its implications.

Design: All cases of chronic hepatitis with "alloimmune" features in HCV-infected, post-LT patients were identified from our files (2006-2011). Inclusion criteria were: positive HCV-RNA with no other viral infections; ≥1 biopsy with PC-rich (>30% PCs) chronic hepatitis with alloimmune features; adequacy (≥5 portal tracts); and no evidence of chronic ductopenic rejection. Clinicopathologic features of each case were re-reviewed. Patient/graft survival were analyzed (Kaplan-Meier and log-rank).

Results: Eighteen cases were included (mean age 57.6 y; M:F= 12:6; mean post-LT time 24.7 m). The table shows the clinicopathologic data. Patient/graft survival compared to the general rHCV post-LT population and a subgroup of these patients diagnosed with acute cellular rejection (ACR) Banff ≥5 (figure).

HAI score*	9.4 (6-13)
% of portal PC*	37.2 (30-70)
PC clusters †	17/18 (94.4)
Portal eosinophils †	3/18 (16.6)
Bile duct injury †	6/18 (33.3)
Portal endotheliitis †	4/18 (22.2)
Central perivenulitis †	12/18 (66.6)
Rosettes †	3/18 (16.6)
Centrilobular necrosis †	10/18 (55.5)
Stage*	1.9 (0-3)
Antinuclear antibody †	9/11 (81.8)
High serum IgG †	6/6 (100)
HCV RNA negative †	6/9 (66.6)
Decrease in immunosupression †	11/18 (61.1)

*mean (range); † pos/total (%)



Conclusions: Our data further supports that post-LT PCH likely represents a variant of late-onset rejection rather than rHCV based on the presence of other features of immune-mediated injury (central perivenulitis), high IgG, as well as recent decrease in dose of immunosuppression and negative HCV-RNA in most patients. We also confirm the generally poor prognosis associated with these cases, comparable to rHCV patients diagnosed with ACR Banff≥5.

1764 Follicular Cholecystitis: Reappraisal of Incidence, Definition and Clinicopathologic Associations in an Analysis of 2413 Cholecystectomies

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Background: Follicular cholecystitis (FC) is considered a distinct type of chronic cholecystitis (CC), described initially in patients with salmonella infections. Most studies are limited to case reports or small series. There is no uniform definition, reported incidence ranges from 0.08-8%, and clinicopathologic characteristics are largely undocumented.

Design: 2413 cholecystectomy specimens were analyzed [1348 for non-obstructive pathologies (1138 primary cholecystitis; 210 non-neoplastic polyps);118 for obstructive pathologies (62 distal CBD/pancreatic tumors, 21 autoimmune pancreatitis and 35 primary sclerosing cholangitis), and 947 for in-situ/invasive gallbladder (GB) neoplasms] for prominent lymphoid follicle (LF) formation. FC was defined as 3 LFs per cm of GB tissue.

Results: FC was seen in 44/2413 cholecystectomies (2%). The frequency was similar in non-obstructive, obstructive and GB neoplasm cases (3.4, 2.6, 0.9 %, respectively). When the 34 FC cases in non-neoplastic GBs contrasted with ordinary CC, patients were significantly older (65 vs 49, p<0.0001), but the gallstone frequency (68 vs 70 %), and female:male ratio (3 vs 2.4) were similar. 5/17 had biopsy proven chronic gastritis (2 with activity and 2 with H. pylori). None had lymphoma, parasites or Salmonella. Microscopically, the LFs were the main process; intervening inflammation was minimal; activity (intraepithelial neutrophils) was not common (noted in 8; severe in 1), and likewise for acute and subacute changes (in 3). Average thickness of the wall 4mm (vs 4.8mm in ordinary CC). LFs were predominantly mucosal, with mucosal micropolyps up to 3 mm; however, transmural involvement was seen in 12, and concentrated around vessels and nerves. Average # of LFs identified/case was 22.5 (3-120). Giemsa and immunostain for H.Pylori were negative in 22 analyzed. IgG4-positive plasma cell densities were low (<10/HPF) in 21/24; while 2 had intermediate (10-49) and 1 had high (≥50) levels.

Conclusions: Follicular cholecystitis (FC) constitutes 2% of cholecystectomies. It is seen in significantly older patients, suggesting a deranged immune response, and about a third of the patients reveal a history of biopsy proven gastritis, an issue that warrants further investigation by molecular analysis for organisms. FC does not seem to be associated with any autoimmune conditions (supported by paucity of IgG4 positive plasma cells), malignancies (i.e. follicular lymphoma) or obstructive pathology.

1765 The Hepatitis Aggressiveness Score (HAS): A Novel Histopathologic Classification System for Posttransplant Recurrent Hepatitis C

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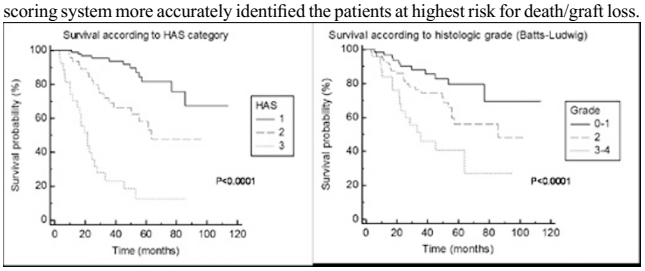
Background: One or more histopathologic features of fibrosing cholestatic hepatitis C (FCH-C) are commonly seen in conventional post-liver transplant (LT) recurrent hepatitis C (rHCV). We investigated the prognostic value of a novel scoring system for rHCV based solely on the evaluation of FCH-like features when applied to the entire group of post-LT HCV-infected patients.

Design: Liver allograft biopsies from 171 rHCV patients were retrospectively evaluated for FCH-C features (mean post-LT time 361 days). The following FCH-related pathologic criteria were assessed: 1- prominent ductular reaction; 2- cholestasis (canalicular and/or intracellular); 3- prominent hepatocyte ballooning with lobular disarray; 4- periportal sinusoidal fibrosis. Patients with chronic rejection, drug toxicity, biliary/vascular complications were excluded. Graft and patient survival were analyzed (Kaplan-Meier and log-rank) based on number of FCH features present in each case.

Results: rHCV cases were classified into 3 categories: type 1- showing 0/4 criteria (57.3% of cases), found to have favorable prognosis; type 2- patients with 1-2/4 criteria (26.9% of cases), with an intermediate prognosis; and type 3- patients with 3-4 criteria (15.7% of cases), showing extremely aggressive rHCV with clinicopathologic features of FCH-C and rapid progression to graft failure or death in 81.4% (22/27) of cases. Patient and graft survivals were statistically different for each group. Compared to the grading system used at our institution (Batts-Ludwig), survival curves by our proposed scoring system more accurately identified the patients at highest risk for death/graft loss.



Conclusions: Histologic rHCV can reliably be classified into 3 prognostic HAS categories: **type 1**, or "typical rHCV", with low mortality/graft failure rate; **type 2**, or "aggressive rHCV", with intermediate mortality/graft failure rate; and **type 3**, or cholestatic hepatitis C, with very high mortality/graft failure rates. Our proposed classification system may be useful in defining FCH-C pathologically and may also be used as a method of stratifying risk of death and graft failure in the entire post-LT rHCV population.

1766 Retransplantation for Fibrosing Cholestatic Hepatitis C (FCH): Outcomes in a Series of 9 Cases

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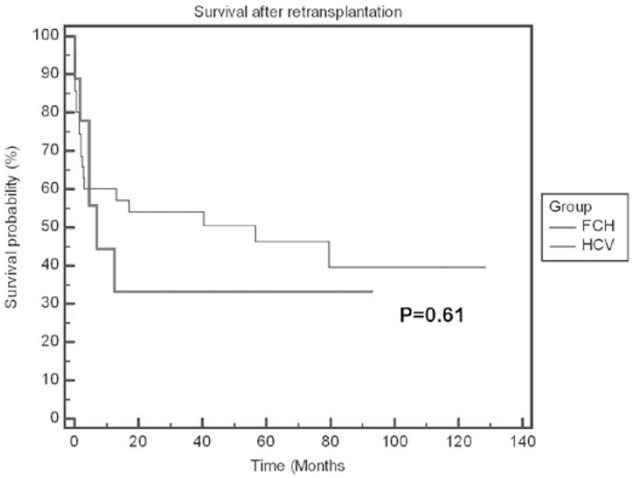
Background: Fibrosing cholestatic hepatitis C (FCH-C) is thought to be associated with significantly worse outcomes compared to non-FCH recurrent HCV (rHCV) and is considered a contraindication for retransplantation (re-LT) in some centers. We documented the clinicopathologic outcomes of 9 patients who underwent re-LT for FCH-C at our institution.

Design: All HCV+ patients diagnosed with post-LT FCH-C undergoing re-LT between 2002 and 2011 were identified from our database. Diagnostic criteria for FCH included cholestatic disease in the absence of biliary obstruction by cholangiography and at least three of the following four histopathologic criteria: 1- prominent ductular reaction mimicking biliary obstruction; 2- marked hepatocyte swelling with lobular disarray; cholestasis (canalicular and/or intracellular); and periportal sinusoidal fibrosis. Clinical and pathologic outcomes were compared to non-FCH rHCV re-LT patients.

Results: Nine FCH-C (M=6, F=3, age 53±9.4) and 35 non-FCH, rHCV patients (M=23, F=12, age 53.2±8.5) underwent re-LT and were included in this study. Significant mortality was seen in the first 2 years post re-LT in both groups. Although our number of re-LT FCH-C patients was small, no statistically significant outcome differences between the two groups could be identified in our series. Clinicopathologic data for each group are shown in Table 1 and Image 1.

	FCH n=9	rHCV n=35	P value
1-year survival post re-LT	44.4%	57.1%	0.26
2-year survival post re-LT	33.3%	45.7%	0.27
Perioperative mortality	11%	20%	0.30
Time to histologic rHCV post-OLT2*	3.63	4.40	0.47
Max. rHCV histologic grade (1st year)#	1.43 (0-4)	1.70 (0-3)	0.65
HCV-related cholestasis (histologic)	55.5%	17.1%	0.09
HCV-related cholestasis (bilirubin)	44.4%	25.7%	0.15
FCH post re-LT	11.1%	3%	0.20
Peak HCV RNA > 30M copies/Dl	22.2%	25.7%	0.37
Biliary complications	11.1%	17.1%	0.24

* mean (months); # average (range)



Conclusions: Our results show high mortality rates in all re-LT HCV-infected patients. However, we were unable to demonstrate worse outcomes in patients undergoing re-LT for FCH-C compared to rHCV. Larger studies are needed to further evaluate outcomes in this setting.

1767 The Overexpression of Enhancer of Zeste Homolog 2 (EZH2) May Be Related to Malignant Behaviors in Intraductal Papillary Neoplasm of the Bile Duct

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Background: Intraductal papillary neoplasm of the bile duct (IPNB), which is regarded as a biliary counterpart of intraductal papillary mucinous neoplasm of the pancreas, usually shows favorable prognosis, but occasionally is associated with invasive carcinoma. The overexpression of polycomb group protein enhancer of zeste homolog 2 (EZH2) is related to an epigenetic suppression of tumor suppressor genes, such as $p16^{INK4a}$, and is reportedly involved in the progression of malignant tumours.

Design: We examined immunohistochemically the significance of EZH2 expression and its association with clinicopathological features, the expression of $p16^{INK4a}$, MUC mucin core proteins (MUC1, MUC2, MUC5AC, MUC5B and MUC6) and p53 in perihilar IPNB with and without invasive carcinoma and extrahepatic IPNB. The subjects included 6 patients with cystic variant of IPNB (cystic type, M/F=3/3, 3 microinvasive and 3 non-invasive,), 6 with IPNB without invasive carcinoma (usual type, M/F=4/2), 11 with IPNB associated with invasive carcinoma (invasive type, M/F=6/5) and 6 with extrahepatic IPNB (ExH-type, all invasive, M/F=5/1). Mucinous cystic neoplasms with ovarian-like stroma were excluded.

Results: The expression of EZH2 was low (labeling index; LI <20%) in all cystic and usual types, whereas it was significantly high in invasive and ExH types ($p<0.05$). The expression of EZH2 was significantly associated with tumor invasion in IPNB ($p<0.01$). The expression of $p16^{INK4a}$ was high (LI >20%) in 3 cystic types (50%), 2 invasive types (18%) and 2 ExH-types (33%). The expression of EZH2 was inversely correlated with the expression of $p16^{INK4a}$ ($p<0.05$). The expression of MUC1 was significantly associated with the degree of tumor invasion in IPNB ($p<0.05$). The expression of MUC2 was more evident in invasive type, especially those associated with mucinous carcinoma ($p<0.05$ versus cystic and usual type). The expression of MUC5AC and MUC5B is evident in all types of IPNB. The expression of MUC6 was significantly more evident in cystic and usual types, compared to ExH type ($p<0.05$). p53 expression was negative (<5%) in all cystic and usual types, whereas p53 expression was higher level in invasive and ExH types ($p<0.05$). The expression of EZH2 was significantly correlated with the expression of MUC1 ($p<0.01$) and inversely correlated with the expression of MUC6 ($p<0.05$).

Conclusions: The over-expression of EZH2 may be related to an upregulation of MUC1 expression and a downregulation of MUC6 expression and may be associated with malignant behaviors in IPNB.

1768 The Deregulated Autophagy of Mitochondria May Be Involved in the Pathogenesis in Primary Biliary Cirrhosis

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Background: We have reported the deregulated autophagy and following cellular senescence characterize the biliary epithelial lesions in primary biliary cirrhosis (PBC). Although it is well known that serum anti-mitochondrial antibodies (AMAs) are detected in most patients with PBC, the significance of AMAs in the pathogenesis of PBC has not been fully clarified, so far. Since mitochondria are major targets of autophagy, we hypothesized that the deregulated autophagy of mitochondria may be related to the autoimmune pathogenesis in PBC.

Design: We examined immunohistochemically the expression of pyruvate dehydrgenase complex-E2 component (PDC-E2), a major target of AMAs, in livers taken from the patients with PBC (n=42) and control livers (n=77) including primary sclerosing cholangitis (PSC) and normal livers. We examined the co-localization of PDC-E2 with microtubule-associated proteins-light chain 3b (LC3), an autophagy marker, p62/sequestosome-1 (p62), a deregulated autophagy marker, and lysosomal-associated membrane protein 1 (LAMP-1) by double immunofluorescence. We also examined

the co-localization of PDC-E2 with autophagy-related markers LC3, p62 and LAMP-1 in cultured mouse biliary epithelial cells (BECs) treated with various stress, such as H_2O_2 (100μM, 2hr), and serum deprivation with and without lysosome inhibitor bafilomycin A.

Results: The expression of PDC-E2 was absent or faint in BECs in small bile ducts in control livers. In contrast, an intense granular and vesicular expression of PDC-E2 was seen in small bile ducts in PBC, especially in the damaged ones. The granular and vesicular expression of PDC-E2 was significantly more frequent in small bile ducts in PBC, compared with control livers ($p<0.01$). The expression of PDC-E2 was co-localized with LC3 and partly with LAMP-1 in the damaged bile ducts in PBC. The locolization of PDC-E2 and p62 was different and not co-localized in BECs in PBC. In cultured BECs, the number of LC3-positive autophagic vacuoles was significantly more increased, when treated with various stress ($p<0.01$). The co-localization of PDC-E2 with LC3-punctae, suggesting the autophagy of mitochondria, was seen in BECs treated with various stress, especially in BECs treated with serum deprivation and bafilomycin A.

Conclusions: The granular and vesicular expression of PDC-E2 was increased in biliary epithelial lesions in PBC and closely related to the deregulated autophagy. The abnormal expression of PDC-E2 associated with deregulated autophagy may be related to the autoimmune mechanism directed to PDC-E2 in PBC.

1769 Low Proliferation/Apoptosis Ratio in Early Biopsies Predicts Adverse Outcome in Cardiac Death Donor Liver Allografts

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Background: Donation after brain death (DBD) has been the most common source for liver used in liver transplantation, however as the demand increases, grafts from donation after cardiac death donors (DCD) are being increasingly used. In comparison to DBD donors, DCD livers are associated with higher rates of graft failure,often presenting as post-transplant biliary strictures. The goal of this study is to identify early histologic predictors of adverse outcomes in DCD donor liver grafts.

Design: Using the Organ Transplant Tracking Record, all DCD and DBD liver transplants from2007-2011 were identified and matched for donor's age, recipient age, warm/cold ischemia time and MELD scores. Patients were divided into 3 groups;DCD donors with graft failure (group1,n=5),DCD(group 2,n=14) and DBD (group 3,n=10) donors with well- functioning grafts. H&E sections were reviewed and immunohistochemical markers of apoptosis (cleaved caspase 3,CC3) and proliferation (Ki-67) were performed on first 3 months post-transplant biopsies. Aperio ScanScope and a nuclear algorithm were used to analyze the nuclear expression of CC3 and Ki-67. IBM SPSS Statistics was used for statistical analysis.

Results: All group 1 biopsies showed persistent ischemic small duct changes, cytoplasmic eosinophilia and lost or disordered epithelium. Only 12.5% of patients in groups 2&3 showed this feature. Anti-CC3 antibody, showed persistent high apoptosis rate in group 1 (mean positive CC3 nuclei: 13.09 ± 9.21 % in group 1; 5.94 ± 5.45 % in group 2(p= 0.04); and 4.70 ± 3.06 % in group 3 (p= 0.01).

Ki-67 showed lower proliferation in group1 than group 2. Positive nuclei in group 1: 3.89 ± 2.76 %,group 2: 10.52 ± 7.87 % (p=0.03). Despite the presence of a trend in group1 vs. 3, it is not statistically significant(p=0.83).The ratio of Ki-67 to CC3 was significantly lower in group 1 than group 2 (0.80 ± 1.49 vs. 2.86 ± 1.82; p=0.02).

Conclusions: DCD grafts with failure showed increased bile duct ischemia and apoptosis but less proliferation in the early post-transplant period compared to matched functioning DCD grafts. Use of apoptotic and proliferative (CC3,Ki-67) markers could be useful in early recognition of high risk DCD grafts which would lead to earlier listing of patient for re-transplant as well as more aggressive treatment of comorbidities such as recurrent hepatitis C.

1770 Amyloidosis of the Liver: Mass Spectrometry-Based Proteomic Analysis Reveals Diverse Etiology Associated with Distinct Histological Features

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Background: Amyloidosis is a protein folding disorder characterized by extracellular accumulation of Congo-red (CR) positive fibrillar deposits. Despite this common histological feature, 28 different proteins have been shown to cause either localized or systemic amyloidosis. Systemic amyloidosis frequently involves the liver, and both the initial diagnosis and typing of amyloid deposits may be challenging. In recent years, mass spectrometry (MS) proteomic analysis has become the gold standard for typing amyloidosis but so far it has not been systemically applied to liver specimens. The aim of this study was to define the pathological features of amyloidosis involving the liver using MS proteomic analysis.

Design: 92 cases of amyloidosis involving the liver were analyzed between 2009-2011. For each case, routine histological sections and CR stains were reviewed, and microdissected fragments of amyloid deposits were analyzed by MS proteomic analysis.

Results: In each case amyloid deposition was confirmed by CR staining. Amyloid deposits had several distinct histological patterns. The most common pattern was peri-sinusoidal (65/92), followed by predominantly portal/globular (22/92), portal/nodular (4/92) and predominantly vascular involvement (1/92). MS proteomic analysis showed that the amyloid deposits were AL-type in 64 cases (30 AL-kappa, 34 AL-lambda), ALect2 in 21 cases, AApoA1 in 4 cases, and ALys, AA and ATTR in one case each. Interestingly, distinct morphological patterns correlated with specific amyloid types. All AL and AA cases had predominantly peri-sinusoidal pattern of involvement, ALect2 and ALys cases had a distinct portal/globular pattern, AAPOA1 cases had a portal/nodular pattern, and the single case of ATTR showed a predominantly vascular pattern.

Conclusions: 1. The etiology of systemic amyloidosis involving the liver is diverse and, in addition to AL and AA amyloidosis include ALect2, AApoA1, ALys and ATTR amyloidosis.

2. ALect2 amyloidosis, recently described as a frequent cause of renal amyloidosis in patients of Hispanic origin, also frequently involves the liver with characteristic portal globular pattern.

3. The histological pattern of liver involvement by amyloidosis provides strong clues with regards to the etiology of the deposits.

4. Given the diversity of amyloid types that could involve the liver, and the differences in management strategies for different types of amyloidosis, typing of amyloid deposits is essential. MS proteomic analysis provides a powerful and comprehensive tool for this purpose.

1771 Immunohistochemical Analysis of Six1 in Hepatocellular Carcinoma: Correlation with Proliferation, Prognostic Parameters, and Outcome

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Background: The incidence of hepatocellular carcinoma (HCC) is increasing, both in the U.S. and worldwide. Homeobox genes encode transcription factors which act as regulators of development. Six1 is one such homeobox gene and is overexpressed in breast, ovarian, hepatocellular, and cervical cancers. Increased Six1 protein expression, detected by Western blot, has been shown to be significantly correlated with pTNM stage, vascular invasion, and poor overall survival.

Design: 135 HCCs in 3 tissue microarrays with 2 1mm cores of each tumor were assessed for Six1 expression by immunohistochemistry (IHC). Stains, scored as intensity 0-3+ and nuclear vs cytoplasmic labeling, were compared to proliferation (mitoses/10HPF, MIB-1 visual mean and high, phosphohistone-3[PPH3] visual mean and high), prognostic parameters (size, grade, stage, vascular invasion, metastases, focality), and outcome (recurrence, survival).

Results: Of the 135 HCCs, 108 (77%) had 2-3+ nuclear Six1 expression, 62 (44%) had 3+ nuclear Six1 expression, 78 (56%) had 2-3+ cytoplasmic Six1 expression, and 30 (21%) had 3+ cytoplasmic Six1 expression.

Significant correlations are summarized below:

Significant Correlations

	Direct Significance	p-value
Six1 nuclear (2-3+)	Mitoses	0.001
Six1 nuclear (3+)	Size	0.01
	Mitoses	0.02
	MIB Visual High	0.001
	MIB Visual Mean	0.001
	PPH3 Visual High	0.003
	PPH3 Visual Mean	<0.001
	Nuclear Grade	0.05
	pT Stage	0.02
	Vascular Invasion	0.03
	Recurrence	see Figure 1
Six1 cytoplasmic (2-3+)	Nuclear Grade	0.04
	Distant Metastasis	0.03
	Recurrence	see Figure 2
Six1 cytoplasmic (3+)	Size	0.002
	Mitoses	0.008
	MIB Visual High	0.03
	Histologic Grade	<0.001
	Nuclear Grade	<0.001
	Recurrence	0.03

Figure 1:

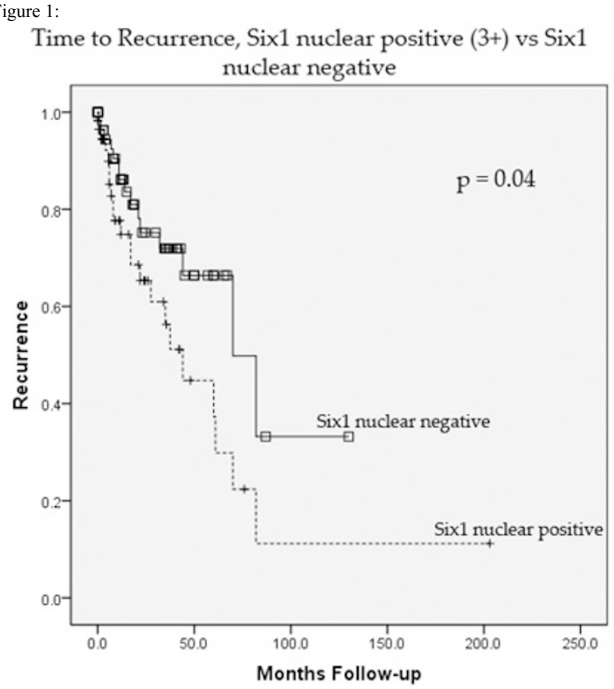
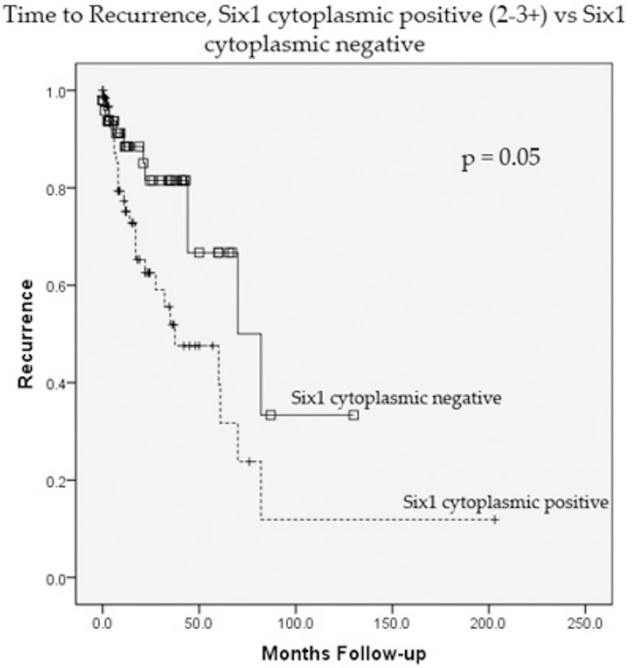


Figure 2:



Conclusions: We demonstrate that strong Six1 positivity by IHC is significantly correlated with increased proliferation, worse prognosis (larger tumor size, higher nuclear grade, histologic grade, and pT stage, and increased vascular invasion and distant metastases), and increased recurrence.

1772 CAP Carcinoma: A Distinct Subtype of Hepatocellular Carcinoma with Unique Morphologic and Molecular Features

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Background: Hepatocellular carcinomas (HCCs) exhibit heterogeneous morphologies. While some morphologies represent variations in growth patterns, others may represent unrecognized variants of HCC with unique clinical and/or molecular features.

Design: Two pathologists independently screened a cohort of 242 unselected HCCs with varying morphologies. Each pathologist divided the cases into separate candidate morphologic variants, and the possible variants were compared. The most consistent candidate variants were further evaluated using both histologic and molecular techniques.

Results: A distinct morphologic variant was characterized by a unique and consistent set of shared histological features: smooth chromophobic cytoplasm, abrupt focal nuclear anaplasia (small clusters of tumor cells with marked nuclear anaplasia in a background of tumor cells with bland nuclear cytology), and scattered microscopic pseudocysts. Therefore, we designate this variant as CAP carcinoma (Chromophobic cytoplasm, Abrupt anaplasia, Pseudocysts). Thirteen cases were identified in the HCC cohort: 6 men and 7 women with a mean age of 59 years. Six of the carcinomas occurred in cirrhotic livers, and alpha-fetoprotein (AFP) was markedly elevated in 6 of the 9 cases for which pre-operative AFP levels were available. There were a variety of underlying liver diseases: hepatitis B (6 cases), hepatitis C (1 case), alcoholic cirrhosis (1 case), cryptogenic cirrhosis (1 case), none (2 cases), and unavailable (2 cases). Based on previous studies that included a small number of CAP carcinomas (before they were recognized as such), we next investigated CAP carcinomas for the alternative lengthening of telomeres (ALT) phenotype. ALT is a telomerase-independent mechanism of telomere maintenance involving homologous recombination which has a distinctive appearance by telomere-specific FISH. ALT is found in approximately 7% of all HCCs. Interestingly, the ALT phenotype was strongly enriched in CAP carcinomas - 11/12 (92%) cases were ALT positive: 9 strongly and 2 weakly ($p<0.0001$).

Conclusions: CAP carcinoma is a newly defined subtype of HCC with unique morphologic and molecular features. Morphologically, it is characterized by chromophobic cytoplasm, abrupt anaplasia, and pseudocysts. On a molecular level, CAP carcinomas exhibit the ALT phenotype. This is the first report of a high prevalence of the ALT phenotype in a morphologically distinct carcinoma subtype.

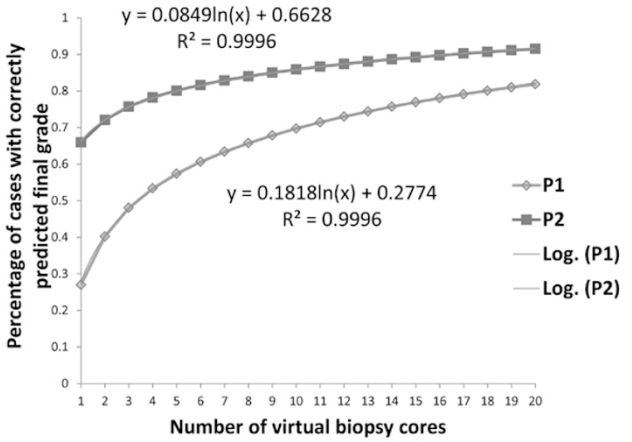
1773 How Many Needle Core Biopsies Are Needed to Comfortably Predict the Histologic Grade of Metastatic Well-Differentiated Neuroendocrine Tumors to the Liver?

Z Yang, LH Tang, DS Klimstra. Penn State Milton S. Hershey Medical Center, Hershey, PA; Memorial Sloan-Kettering Cancer Center, New York, NY.

Background: As determined by Ki67 labeling index (especially from the highest labeling region), the histologic grade of metastatic well-differentiated neuroendocrine tumors (NETs) correlates with patient survival. Substantial intratumoral heterogeneity in Ki67 labeling index exists, resulting in different WHO grades (G1 vs G2). It is of practical importance to determine the optimal number of random needle biopsy cores needed to sample the area with the highest grade.

Design: The question was addressed in a simulated fashion using virtual core biopsies on resection specimens. Immunohistochemical staining for Ki67 was performed on 45 resected metastatic NETs to the liver. The scanned whole slide images were segmented into multiple subsections to simulate needle core biopsies, and the Ki67 labeling index for each subsection was determined using digital image analysis. The final grade of each case was based on the highest labeling region. In heterogeneous cases (containing both G1 and G2 regions) the probability of obtaining that final grade was expressed as the ratio of the number of cores containing the final grade over total number of cores, while in homogeneous cases the probability was 100% by definition.

Results: The cohort consisted of 24 homogeneous and 21 heterogeneous cases. Percentage of cases for which the final grade was correctly predicted on virtual core biopsies increased with the number of cores in a logistic fashion. The percentages of correctly predicted cases in the heterogeneous group (P1) were 27%, 40%, 48%, 53%, and 57% for 1, 2, 3, 4 and 5 cores, respectively. The percentages in the whole cohort (P2) increased to 66%, 72%, 76%, 78% and 80% for the respective number of cores due to inclusion of homogeneous cases. A 90% accuracy required 31 cores in the heterogeneous group, and 17 cores for the whole cohort.



Conclusions: A high degree of accuracy in predicting histologic grade of NETs requires multiple core biopsies particularly in cases exhibiting intratumoral heterogeneity. At least five cores are needed to achieve reasonably acceptable accuracy and may be clinically feasible.

1774 Evaluation of Prognostic Markers in Metastatic Well-Differentiated Neuroendocrine Tumors to the Liver

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Background: Well-differentiated neuroendocrine tumor (NET) is a heterogeneous group. Current WHO classification stratifies those tumors into two histologic grades (G1 and G2) based on mitotic activity or Ki67 labeling index, whose prognostic value has been validated in both primary and metastatic tumors. Additional markers were suggested to have prognostic significance in various primary NETs, but their roles in metastatic disease had not been tested.

Design: Tissue microarray was constructed from 42 surgically resected metastatic well-differentiated NETs to the liver. Immunohistochemical staining for $p27^{Kip1}$, CK19, CD117, PAX8 and CD99 was performed and the staining was scored in a semiquantitative manner. The positivity of each marker was compared to the histologic grade determined previously by Ki67 labeling index, and their prognostic significance was analyzed using the Kaplan-Meier estimate.

Results: Most metastatic NETs were positive for $p27^{Kip1}$ (97.6%); while negative for PAX8 and CD99 (92.9% negative in each). A subset of the tumors showed positive staining for CK19 (64.3%) or CD117 (33.3%). We found no correlation between the positivity of any of the above markers and the histologic grade based on Ki67 labeling index. Upon Kaplan-Meier survival analysis, there was no difference in patient survival based on the expression of $p27^{Kip1}$, CK19, PAX8 or CD99. CD117-negative patients showed slightly better overall survival than CD117-positive ones (70.8% vs. 40.4% five-year survival; or 103 vs. 47 months of median survival); however, the difference did not reach statistical significance ($p=0.16$).

Conclusions: Although $p27^{Kip1}$, CK19, CD117, PAX8 and CD99 may have prognostic significance in some primary well-differentiated NETs, no correlation with patient survival or tumor grade was found in metastatic tumors. The small survival difference between CD117-negative and CD117-positive groups seen in our study needs to be further tested in a larger cohort.

1775 Additional Morphological Features of Hepatocellular Carcinoma (HCC) Correlate with Disease Free Survival (DFS) but Are Not Independent Prognosticators: A Multivariate Survival Analysis

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Background: In addition to the routinely reported gross and microscopic morphological features of HCC, multiple other findings, such as histologically identifiable tumor capsule (TC), tumor capsule invasion (TCI), tumor infiltration by neutrophils (TIN),

tumor infiltration by lymphocytes (TIL), apoptotic hepatocytes (AH), overall number of apoptotic cells (AC), intratumoral fibrosis (ITF), mitotic rate (MR) and gross tumor classification (GTC) have been described in literature as independent prognosticators of recurrence and metastasis after curative resection. We analyzed macro- and microscopic findings in HCC as prognostic factors of DFS after orthotopic liver transplantation.

Design: This study is a retrospective analysis of liver explant specimens with HCC and a corresponding chart review. 90 consecutive cases with a minimum 27-month follow up were identified in our departmental files from 2004-2009. The following histological features were evaluated on slide review: Edmondson-Steiner grade, margin status (MS), small vascular invasion (SVI), large vascular invasion (LVI), TCI, TIN, TIL, AH, AC, ITF and MR. The size of the largest nodule (SLN), number of nodules (NN), presence of satellite nodules (SN), necrosis or hemorrhage (NH), laterality, involvement of both lobes (BL) and GTC were recorded based on gross description and archive photos. The significance of individual parameters was established using Kaplan-Meier survival analysis (KMSA). As a second step, statistically significant findings were analyzed using a Cox regression multivariate survival analysis (CRMSA) model.

Results: The median age of patients was 57 years. Tumors ranged from grade 1 to 4, AJCC stage T1 – T4b. 20 patients (22%) had documented recurrence (either intra- or extra-hepatic) with 14.8 months of median disease free survival (DFS). Based on KMSA method, TCI, TIL, AH, MR, NN, SLN, HN, LVI, SVI, MS, BL and GTC were individually statistically significant. These values were subjected to CRMSA. Only size of the largest nodule, MS, LVI and number of nodules were identified as independent prognostic factors of DFS.

Conclusions: To our knowledge, this is the first multivariate survival analysis study which included routinely reported and additional morphological features of HCC. Multiple individual morphological features of HCC were shown to have significant correlation with clinical outcomes after liver resection. However, only features included in the current AJCC staging scheme had prognostic significance for DFS based on a CRMSA model.

1776 Aberrant von Willebrand Factor Expression of Sinusoidal Endothelial Cells and Quiescence of Hepatic Stellate Cells Help in the Diagnosis of Hepatoportal Sclerosis

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Background: Hepatoportal sclerosis (HPS) is an under-recognized disease of uncertain etiology and the diagnosis can be easily missed on needle liver biopsy. HPS is characterized by portal fibrosis and obliteration of small and medium branches of the portal vein, resulting in the development of portal hypertension, and its sequelae. A recent FDA advisory about the use of anti-retroviral agents (ddI) as a cause of non-cirrhotic portal hypertension in HIV+ individuals has been issued. Liver sinusoidal endothelial cells (SEC) are unique because expression of CD34 and von Willebrand factor (vWF) are only found in periportal areas. Hepatic stellate cells (HSC) when activated lead to liver fibrosis and their activation as characterized by alpha smooth muscle actin (ASMA) expression, is unknown in HPS. We sought to find immunomarkers that might help in making the diagnosis of HPS.

Design: Immunohistochemical (IHC) staining for CD34, vWF and ASMA was performed and evaluated in 10 (3 core biopsies and 7 explants) clinically and histologically well-characterized HPS liver specimens.

Results: As is typical for HPS, the pathological findings were heterogeneous, but all specimens showed various degrees of dense portal fibrosis and obliterated portal veins. Abnormally dilated channels were present in the vicinity of portal areas. CD34 (+) staining was mainly confined to small vessels in the portal tracts and SECs at periportal areas, a similar finding in normal livers. Unlike CD34, however, SEC expression of vWF was (+) in a patchy or geographic pattern and was particularly prominent in perivenular areas. HSCs were not activated as defined by totally absent staining for ASMA in all HPS cases.

Conclusions: In this study, we found that immunostaining for CD34, vWF and ASMA may aid in the diagnosis of HPS. CD34 and vWF are commonly used endothelial markers; vWF mediates platelet adhesion to the subendothelium at sites of vascular injury as well as binding to and stabilizing factor VIII in the circulation. The current study shows a patchy and geographic SEC(+) pattern for vWF, suggesting that endothelial injury plays a role in the pathogenesis of HPS. HSCs are not activated in HPS suggesting that these cells are not directly involved in the pathogenesis of HPS. The aberrant expression of vWF may thus aid in the diagnosis of HPS, particularly when confronted with otherwise apparent normal liver histology on needle biopsy.

1777 Expression of Calretinin and CK5/6 in Cholangiocarcinoma

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Background: One of the mesothelial lineage markers, mesothelin, has been found expressed in, and a potential treatment target of, cholangioacarcinoma (CC). It raises the possibility that CC may be derived from cells sharing or switching to mesothelial phenotype. However, the expression of other mesothelial markers in CC is largely unknown.

Design: Twenty three well documented CC cases were retrieved from the archive of our institution. Immunohistochemical study of Calretinin (DC8), WT1 (6F-H2), D2-40, CK5/6 (D5/16 B4) and CK19 (b170) was done on formalin fixed paraffin embedded sections from each case using standard immunohistochemical protocols. We compared the protein expression levels between CC and normal bile duct (NBD) on the same block.

Results: All of the CC and BD are positive for CK19 (23/23), and all or mostly negative for WT1 (0/23) and D2-40 (1/23). Interestingly, calretinin immunoreactivity was detected in 52.2% (12/23) of CC, but not in NBD (0/23). CK5/6 was also detectable in 73.9% (17/23) of CC as well as all NBD (23/23) (Table).

Expression of Calretinin and CK5/6 in cholangiocarcinoma

	Calretinin		CK5/6	
	CC	NBD	CC	NBD
Positive (total)	12 (52.2%)	0	17 (73.9%)	23 (100%)
1+	4 (17.4%)	0	6 (26.1%)	2 (8.7%)
2+	4 (17.4%)	0	4 (17.4%)	6 (26.1%)
3+	4 (17.4%)	0	7 (30.4%)	15 (65.2%)
Total	23 (100%)	23 (100%)	23 (100%)	23 (100%)

CC: cholangiocarcinoma; NBD: normal bile duct; 1+: 1-25% cells stained; 2+: 26-75% cells stained; 3+: >76% cells stained.

Conclusions: Significant numbers of CC could express calretinin and CK5/6, two of the commonly used mesothelial markers. Calretinin and CK5/6 immunoreactivities should be interpreted with caution in cases with differential diagnoses of mesothelioma or adenomatous tumor versus CC (either metastatic or primary). A full mesothelioma panel, including WT1 and/or D2-40 as shown in this study, should be used to better define a mesothelial lineage. The biology of this partial mesothelial phenotype in CC is unclear but might also be useful in differentiating CC from other adenocarcinomas.

1778 Giant and Small Cavernous Hemangiomas of the Liver: A Clinicopathologic Comparison Including Hemangioma-Like Vessels

Q Zhao, M Taggart, SC Abraham. MD Anderson Cancer Center, Houston.

Background: Cavernous hemangioma (CH) is the most common benign liver tumor. A recent publication described several underrecognized features of CH, including the presence of hemangioma-like vessels (HLVs) in the liver parenchyma adjoining giant CHs (Liver Int. 2006;26:334-8). The authors of that study postulated that HLVs might be a mechanism of CH expansion, as they were very common (15 of 19, 79%) around giant CH. However, CHs of smaller sizes were not studied and it is unknown if HLVs also occur in small CH and whether differences in HLV density might correlate with size and multiplicity of hepatic CH.

Design: We studied 28 patients with giant CH (>4 cm) and 56 patients with small CH (≤4 cm). Age, gender, and multiplicity of hepatic CH were recorded for each patient. For each CH, we determined the presence, number, greatest size, and distance of HLVs from the CH-liver interface. Because of the varying numbers of histologic sections of CH-liver interface taken for each case, we also calculated an HLV 'density' by dividing the total number of HLVs by the number of sections containing CH-liver interface (e.g., a CH with 2 HLVs in a total of 2 sections had a density of 1).

Results: Patients with giant CH were significantly younger than those with small CH. They were more likely to have multiple hepatic CH, but this did not reach statistical significance. HLVs were significantly more frequent around giant CH, occurred in greater numbers, and could be identified at greater distances from the CH-liver interface. Even when normalized for number of available histologic sections, giant CH demonstrated a significantly greater HLV density.

Clinicopathologic Comparison of Giant and Small Cavernous Hemangiomas (CH)

	Giant CH	**Small CH**	**p**
Age	49 (28-79) yr	55 (34-83) yr	0.02
Sex (female)	57%	61%	NS
Multiple CH	54%	38%	NS
HLVs present	81%	32%	<0.001
HLV density	2.83	0.47	<0.001
HLV size	≤3 mm	≤2.2 mm	--
HLV number	≤200	≤25	--
HLV distance from CH	≤4 cm	≤0.7 cm	--

* 26 giant CH from 28 patients and 71 small CH from 56 patients

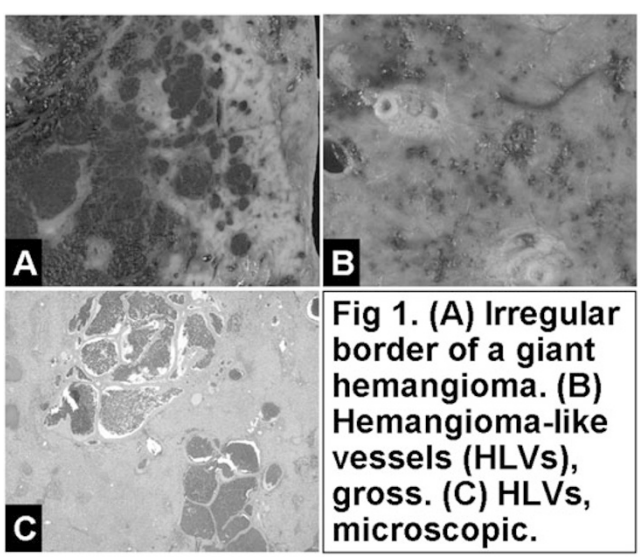

Fig 1. (A) Irregular border of a giant hemangioma. (B) Hemangioma-like vessels (HLVs), gross. (C) HLVs, microscopic.

Conclusions: Our study demonstrates significant clinicopathologic differences between patients with giant and small CH, especially in the prevalence, density, and maximum extent of HLVs. These differences support a role for HLVs in the growth and eventual size of hepatic angiomas.