Liver

1654 Relapsed and Metastatic Hepatoblastoma: A Comprehensive Genomic Profiling Study

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Background: Although hepatoblastoma (HBL) is often cured by combinations of surgery and chemotherapy, relapsed and refractory HBL are a rare cause of progressive metastatic disease in pediatric oncology patients.

Design: DNA was extracted from 40 microns of FFPE specimen from 31 cases of relapsed, refractory and metastatic HBL. Comprehensive genomic profiling (CGP) was performed using a hybrid-capture, adaptor ligation based next generation sequencing assay to a mean coverage depth of >672X. Tumor mutational burden (TMB) was calculated from a minimum of 1.11 Mb of sequenced DNA as previously described and reported as mutations/Mb. The results were analyzed for all classes of genomic alterations (GA), including base substitutions (sub), insertions and deletions (short variants; SV), fusions, and copy number changes including amplifications (amp) and homozygous deletions.

Results: The 31 HBL patients had a mean age of 6.4 years (range 2-17 years); 17 (55%) were male and 14 (45%) were female. There were 6 (29%) pure embryonal, 16 (52%) mixed fetal and embryonal, 3 (10%) pure fetal, 3 (10%) mixed mesenchymal, 2 (6%) macrotubular and 1 (3%) small cell undifferentiated subtypes. CGP was performed on liver biopsies (48%), liver resections and total hepatectomies (19%) and metastasis biopsies (32%). All patents had relapsed or metastatic disease at the time of CGP. The 31 HBL featured 1.84 GA/case. CTNNB1 was by far the most frequent GA seen in 19 (61%) of cases. All 3 (100%) of the mixed mesenchymal HBL had *CTNNB1* sub. There was no significant further correlation of GA with HBL histologic subtype. In additional to the potential targeting of *CTNNB1*, other rarely identified possible targetable GA included *ERBB4* (6%) and *FBXW7*, *SRC* and *BRCA2* (each at 3%). The mean TMB was 3.5 mut/Mb, the median was 1.7 mut/Mb. There were 2 HBL with $\geq$ 10 mut/Mb and 1 HBL with $\geq$ 20 mut/Mb.

Conclusions: Relapsed and metastatic HBL is characterized by a general paucity of GA and is dominated by the greater than 60% frequency of *CTNNB1* mutations. Although the mixed mesenchymal variant of HBL appears to always feature a *CTNNB1* mutation, no significant differences in genomic landscape could be seen among the various histologic subtypes. Although potentially targetable GA are seen on occasion in HBL and a small number of cases have high TMB with potential to respond to immune checkpoint inhibitors, based on the current data, it appears that going forward relapsed and refractory HBL will remain a challenge for the development of novel therapies.

1655 ABHD 6 Immunostain Identifies a New Molecular Pathway Implicated in Human Hepatocellular Carcinoma Pathogenesis

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Background: ABHD6 is a member of the α/β hydrolase domain enzyme family16, and acts as an intracellular lipase mediating the turnover of several classes of signaling lipids. Preliminary studies in mice have shown that ABHD6 inhibitors prevent the turnover of a novel class of tumor suppressive 1-MAG lipids that signal through PPARα to blunt the progression of viral- and obesity-driven hepatocellular carcinoma (HCC). We hypothesize that ABHD6 plays a role in the pathogenesis and tumor progression of human HCC as well.

Design: After IRB approval, a cohort of HCC and control cases were identified in a clinical databases (2005-2009). Clinical information was obtained from chart review. H&E stained slides were reviewed for pathology data. The tumors were stained with ABHD6 (New England Biolabs, rabbit polyclonal, dilution 1:75). Stain qualitative and quantitative assessment was done (strong cytoplasmic staining at 4 x: positive; > 50% positive: diffuse and 5-50% positive: patchy).

Results: A total of 25 HCC from 24 patients , 20 males, average age: 66 years (range: 55-78). All patients had cirrhosis of diverse etiologies (4 non-alcoholic steatohepatitis, 6 alcoholic steatohepatitis, 10 hepatitis C, 2 hepatitis B, 2 primary biliary cholangitis). Based on the morphologic assessment 6 tumors were well, 14 moderately and 5 poorly differentiated. Lymphovascular invasion (LVI) was identified in 5/25 and positive margins in 1/25 cases. On average, patients had 2 tumors with a size of 2.51 cm (range: 1-6.3). ABHD6 stain was positive in 24/25 tumors , 21 with diffuse pattern. Non-neoplastic cirrhotic liver (control) was negative in 27/28 cases. All HCC with LVI and poorly differentiated grade revealed diffuse ABHD6 expression. Four patients developed recurrent disease (mean follow up: 52 months (3-120)), independently of ABHD6 expression.

Conclusions: ABHD6 stain is involved in the pathogenic mechanism of tumorigenesis in human HCC. We identified a definitive differential ABHD6 expression between HCC and cirrhosis independently of the underlying etiology ($p<0.0001$), which makes this an interesting target for potential therapeutic interventions. Diffuse ABHD6 staining was noted in HCC with poorly differentiated component and lymphovascular invasion, although this was not statistically significant possibly due to the relatively small sample size.

1656 Hepatic Involvement in Hemophagocytic Lymphohistiocytosis (HLH)

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Background: Patients with HLH often have abnormal liver chemistry tests and frequently undergo liver biopsy to obtain histologic confirmation of the diagnosis. However, hemophagocytosis is difficult to identify in liver specimens and few studies have systematically analyzed the morphologic findings in these cases. Since HLH can occur in association with a range of diseases and is being diagnosed with increasing frequency, further study of the pathologic findings in liver specimens is warranted.

Design: Cases of clinically suspected HLH with liver involvement were identified from the pathology files of 5 institutions. H&E and IHC slides were reviewed. IHC for CD163 was performed in 9 cases with unstained slides available and in 3 cases of non-HLH-associated lobular hepatitis.

Results: Fourteen specimens (9 biopsies, 4 autopsies) were identified from 13 patients ranging in age from 6 days to 77 years. Eleven patients were male. Two patients had familial HLH, 6 patients had a hematologic malignancy, 2 patients had a rheumatologic condition, and 4 patients had a viral infection without other predisposing conditions. One biopsy was taken following HLH treatment. A sinusoidal lymphohistiocytic infiltrate was present in all 14 cases, and most cases (64.3%) also displayed a portal lymphohistiocytic infiltrate. Hemophagocytosis was identified in 10 cases (71.4%). Eight cases (57.1%) exhibited bile duct damage, with infiltration of bile duct epithelium by lymphocytes in 6 cases. Endotheliitis was present in 6 cases, including 5 cases with lymphocyte-mediated bile duct damage. Hepatocellular injury was evident in 10 cases (71.4%). IHC for CD163 revealed diffuse Kupffer cell staining in all cases; in 7/9 cases (77.8%) CD163 also highlighted plump sinusoidal macrophages, which were often present in small clusters. In contrast, lobular hepatitis cases exhibited CD163+ Kupffer cells but no plump or clustered sinusoidal CD163+ macrophages.

Conclusions: In addition to a prominent sinusoidal and portal lymphohistiocytic infiltrate with hemophagocytosis, a subset of HLH cases display lymphocyte-mediated bile duct damage and endotheliitis, as previously described in familial HLH. While staining of Kupffer cells with CD163 is not specific for HLH, the presence of CD163+ plump sinusoidal macrophages, often in clusters, appears to be characteristic of this condition.

1657 Is Albumin RNA In Situ Hybridization (RISH) a Reliable Marker for Intrahepatic Cholangiocarcinomas?

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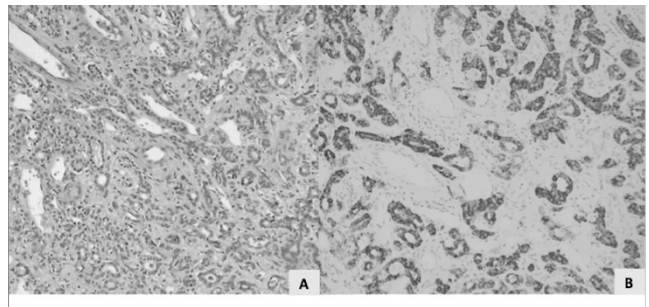
Background: Intrahepatic cholangiocarcinomas (ICCs) show morphologic diversity, ranging from tumors composed of non-mucinous small ducts, mucin-producing large duct tumors and tumors with mixed hepatocellular carcinoma (HCC) features. Diagnosing ICCs can be difficult, especially on biopsy, not only due to the morphologic diversity, but also because metastatic tumors are often in the differential diagnosis. Recently, albumin ISH has been shown to be a potential sensitive and specific marker for ICC with 99% sensitivity (PMID #25519926). Using a different ISH technology (RNAscope, RNA in situ hybridization, RISH) we evaluated the expression of albumin ISH in ICC.

Design: We evaluated 31 ICCs in a triplicate TMA; 2 normal liver tissue cores served as controls. We performed RISH for albumin using the RNAscope detection kit (Leica Biosystems, Buffalo Grove, IL) with the Bond III autostainer, using probe Hs-ALB-01 (Advanced Cell Diagnostics, Newark, CA). A negative probe (Dap B) and a positive probe for RNA (PP1B) were also included. A semiquantitative method of scoring was used: 0=no positive cells; 1= <5% tumor cells; 2 = 5-50% tumor cells and 3= > 50% tumor cells. Staining intensity was also scored as 0=negative, 1=weak, 2= moderate, 3= strong. Dot like cytoplasmic reactivity with at least 2+ intensity (any %) or >5% tumor cells (any intensity) in at least one core was considered positive.

Results: Albumin RISH was positive only in 14 of 31 (45%) ICCs. 5 of 6 (83%) of our combined HCC-CC were positive in the CC component. None of the tumors with mucin production were positive (0/6).



Albumin RISH positive staining in ICC: A. H&E, 200X. B. Albumin RISH, 200X.

Conclusions: In our cohort, albumin RISH showed a sensitivity of 45% in ICCs, supporting the morphologic diversity of ICCs. Albumin RISH does not appear to be a highly sensitive marker for ICC and hence cannot be used as a stand alone marker. However, it may be helpful when included in a panel of immunohistochemical stains to differentiate ICC from other tumors, such as metastatic carcinomas from pancreas, extrahepatic biliary tree and gastrointestinal tract that have been shown to be negative for albumin ISH.

1658 Liver Histology After Hepatitis C Virus Treatment with Sustained Virologic Response: Should We Expect Inflammation?

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Background: Since the introduction of therapies for the treatment of Hepatitis C virus (HCV), especially with new direct acting antiviral agents, sustained virologic response (SVR: aviremia 24 weeks after therapy completion) is the new norm. In this new era of HCV management, the pathology of the liver after SVR is not well characterized and could provide new insights concerning thresholds for inflammatory activity in this population. Our aim is to study the histologic features of liver posttreatment for HCV.

Design: 35 patients with liver biopsies or transplants performed after HCV treatment were retrieved from the pathology database (2000 – 2016). 16 patients (19 liver samples: 10 posttreatment explants, 6 posttreatment biopsies, and 3 corresponding pretreatment biopsies) met our inclusion criteria (HCV SVR with treatment history and absence of clinical/histologic confounding factors; e.g. steatosis and coinfection). Posttreatment liver histology was scored for inflammatory activity and fibrosis by the Ishak scoring system for chronic hepatitis. Unpaired t-test was used for statistical analysis.

Results: Pretreatment biopsies show higher total inflammation scores (mean 4 out of 18, range 3-6) compared with posttreatment (mean 2 out of 18, range 0-5). Portal lymphoid aggregates are more common in the posttreatment setting (12 of 16) compared with pretreatment biopsies (0 of 3). Dividing posttreatment samples into less than 5 months (n=8, mean 1.1 months, range 0-3) and 5 or more months (n=8, mean 19 months, range 5-72) after first negative HCV serology shows a significant decrease in activity scores over time (p-value 0.034). Concerning fibrosis, posttreatment explants showed advanced fibrosis (Ishak 5 or 6 out of 6): thin septa with features of regression in 4, wide septa in 4, and mixed thin/wide septa in 2.

Conclusions: Histologic features similar to mildly active HCV infection persist after SVR. These findings may be useful when evaluating liver biopsies in this population, to consider other possible causes of inflammatory activity. However, inflammation post-SVR raises the possibility of persistent HCV infection not detectable by serologic tests. Further study is needed, including PCR testing of posttreatment liver samples to rule out potential occult HCV. Our findings also suggest inflammation may decrease and eventually resolve long after SVR. This study is limited by a small sample size due to a diminishing number of biopsies performed for HCV and follow-up after SVR, which appears to be a widely experienced practice shift not unique to our institution.

1659 Non-Alcoholic Fatty Liver Disease in Patients with Inflammatory Bowel Disease and Primary Sclerosing Cholangitis

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Background: Non-alcoholic fatty liver disease (NAFLD) occurs in patients with inflammatory bowel disease (IBD). Susceptibility to NAFLD is related to the metabolic syndrome and insulin resistance, as well as malabsorption, higher prevalence of bowel resection, and likely gut microbiotal dysbiosis. Liver complications from multiple causes, including NAFLD and primary sclerosing cholangitis (PSC) contribute to treatment and prognosis of patients with IBD. However, the potential interplay between liver disease processes in this population remains understudied.

Design: We retrospectively assessed presence and severity of steatosis and steatohepatitis in liver specimens from 49 patients with IBD only, 42 with IBD and co-morbid PSC, and 26 with IBD and PSC after orthotopic liver transplantation. Steatosis was quantified by percentage and steatohepatitis was evaluated according to NASH-CRN criteria. Clinical, laboratory, and imaging findings were correlated using Student's t-test, univariate linear least squares regression, and multivariate regression modeling.

Results: Among liver specimens, patients with IBD had higher prevalence of at least grade 1 steatosis (57%) than patients with IBD and PSC (14%), or IBD and PSC post-transplant (7%) ($p < 0.001$). The average percentage of steatosis was 25% +/-8% (95% C.I.) for IBD only, 4% +/-2% for co-morbid IBD and PSC, and 2% +/-1% for IBD and PSC post-transplant ($p < 0.001$). Prevalence of steatohepatitis was significantly higher in the IBD only population (14%) than in the IBD and PSC +/- transplant groups (0%) ($p=0.01$). Despite these differences in susceptibility to NAFLD, the three populations had statistically indistinguishable average BMI and total cholesterol and prevalence of hypertension, diabetes, and alcohol use. Multivariate regression modeling of clinical and laboratory data revealed BMI, hypertension, and diabetes as significant correlates to NAFLD severity in all studied populations.

Conclusions: Patients with co-morbid IBD and PSC have significantly less susceptibility to NAFLD and NASH than those with IBD alone, despite similar prevalence of major NAFLD risk factors (obesity, diabetes, hypertension, hyperlipidemia). The lower frequency of NAFLD in this population persists following liver transplantation. These findings suggest that factors not intrinsic to the liver and substantially protective against NAFLD are present in patients with co-morbid IBD and PSC, as compared to patients with IBD only. Candidate factors include medications, underlying genetics, and gut microbiome alterations.

1660 Comparison of Donation After Cardiac Death (DCD) Liver Transplant Recipients with Hepatitis C (HCV) and Non-Hepatitis C Related Cirrhosis

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Background: Liver transplantation (OLT) using DCD donors is becoming increasingly necessary, but histologic features of DCD OLT are not well defined.

Design: Histologic findings in liver biopsies (n=92) from DCD donors in recipients with HCV (n=60) and non-HCV related liver disease (n=32) were reviewed in a blinded fashion. Biopsies were subgrouped into time 0, 0-6 months (m), and >6 m.

Results: The most common pre-OLT diagnoses in the non-HCV group were cryptogenic cirrhosis (30%), alcoholic steatohepatitis (17%), and non-alcoholic steatohepatitis (13%). DCD donors were similar ages (30 vs 26) and had similar warm and cold ischemia times. At time 0, zone 3 necrosis ($p<0.01$) and bile duct injury ($p<0.01$) were more common in non-HCV group. Reperfusion injury was diagnosed equally in both groups. HCV patients (pts) had more biopsies during the follow up period (Table 1) and showed features of HCV re-infection. At 0-6 m, pericholangitis was noted more in non-HCV (table 1). Acute cellular rejection (ACR, $p=0.04$) and findings of biliary obstruction ($p=0.02$) was seen more in the non-HCV pts.

At >6 m, bile duct injury were seen in both cohorts (67% and 87%). Pericholangitis was slightly increased in non-HCV (Table 1). Features of biliary obstruction were noted more in non-HCV ($p<0.05$). Mild ACR (25% vs 0%) was seen more in non-HCV, whereas HCV had more chronic rejection (5% vs 0%). 1 year graft survival was similar between the two groups. The overall 5 year patient (87% vs 91%) and graft survival (79.3% vs 85%) were better in the non-HCV group.

Table 1

	Time 0		Follow-up 0-6 m		Follow-up >6 m	
	HCV/ Non-HCV	p-value	HCV/Non-HCV	p-value	HCV/Non-HCV	p-value
Total Biopsies	16/18		23/6		21/8	
Bile duct injury (%)	19/83	<0.01*	74/100	0.2	67/87	0.3
Pericholangitis (%)	0/17	0.08	30/50	0.4	19/37	0.3
Zone 3 Dropout (%)	56/94	<0.01*	79/50	0.02*	14/0	0.2
Mild ACR (%)	0/0	-	4/33	0.04*	0/25	0.02*

Conclusions: Features of bile duct injury and parenchymal ischemia were seen more in the non-HCV DCD pts raising concern for vulnerability to biliary injury. Though 1 year graft survival between the groups was similar suggesting graft recovery. While not significant, non-HCV pts did better long-term, albeit all HCV pts had recurrence of HCV. Given the era of new HCV therapy, these findings indicate that DCD donors are a viable option for a wide range of recipients.

1661 Hepatocellular Neoplasms Arising in Genetic Metabolic Disorders: Steatosis Is a Common Finding in Both Tumor and Background Liver

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Background: Genetic metabolic disorders can lead to hepatocellular adenoma (HCA) or carcinoma (HCC). Due to their rarity, these cases have not been systematically studied. We aim to characterize their clinical and pathologic features.

Design: Hepatocellular neoplasms arising in genetic metabolic disorders were retrospectively searched from our centers. Demographic data, clinical history and pathology including immunohistochemistry were reviewed. Patients with viral hepatitis, obesity, and hormonal or excessive alcohol uses were excluded.

Results: 9 cases were identified, with a moderate female predominance (67%). The age at presentation ranged from 5 months to 32 years. Five patients had GSD type 1 (M:F=1:4, age ranged 3 to 32 years): 3 developed HCA, all multiple (3 to >15); and 2 developed HCC. One patient had ornithine carbamyl transferase deficiency (OCTD, 17 y/o girl), who developed a HCA. None of these 6 patients had cirrhosis, although 3 of the GSD/HCA patients had bridging fibrosis. Among the remaining 3 patients, 2 had tyrosinemia (5 m/o and 2 y/o, both boys), and one had Navajo neurohepatopathy (9 y/o girl), who all developed multiple HCCs (>5 in the tyrosinemia patients and 2 in the Navajo neurohepatopathy patient) in a background of cirrhosis. Two GSD/HCA patients were of the inflammatory subtype. The other GSD patient with HCA had the unclassified subtype. The OCTD patient had inflamatory HCA. One GSD patient had multiple HCCs (16). The other GSD patient with HCC had solitary HCC. The sizes of HCAs ranged from 0.2 to 5 cm and HCCs ranged from 1.4 to 9 cm. All HCCs were well-differentiated. Most patients (7, 75%) had mild to severe steatosis in tumors. Steatohepatitic HCCs were observed in the GSD/multiple HCC patient. The background liver showed mild to severe steatosis in most (8, 89%) patients. Most patients (8, 89%) received liver transplantation. All were alive without tumor recurrence at follow up.

Conclusions: Genetic metabolic disorders can lead to HCA or HCC. The most common in our series is GSD, followed by tyrosinemia. Most patients present with multiple tumors. Liver transplantation was performed in most cases without tumor recurrence. When young patients without obesity or hormonal/alcohol uses present multiple HCA/HCCs showing steatosis in both tumor and background liver, workup for possible genetic metabolic disorder should be considered. Steatosis may contribute to tumorigenesis in these cases.

1662 Metastatic Paragangliomas & Pheochromocytomas in Liver Are Challenging to Diagnose

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Background: Paragangliomas are rare tumors that arise from neural crest-derived endocrine cells distributed along sympathetic and parasympathetic nerves. Based on the consult practices of two referral medical centres, we have noticed that these tumors are frequently misdiagnosed when metastatic to the liver. This diagnosis can be challenging because there is little or no published data on the findings of metastatic paragangliomas and the histology can mimic other tumors; the immunohistochemical

profiles overlap with other tumor types; and liver metastases may occur after long periods of remission. To further clarify the histological features, we reviewed specimens with known diagnoses.

Design: We reviewed the histological findings in specimens of known metastatic paragangliomas to the liver seen at a large referral center. A control group of neuroendocrine tumors (NET) metastatic to the liver was examined, as NET tumors are the principal diagnostic pitfall.

Results: Paraganglioma specimens included two biopsies and six excisions. The median age at biopsy or surgery was 23 (range 14 to 36). On histology review, paragangliomas had several morphologic features that were more common than in NETs: very prominent micro-/ macrovascularity (7/7 paragangliomas); lack of trabecular architecture (7/7); and clear cell change/ cytoplasmic foaminess (7/7). Other findings in the paragangliomas that could also be seen in NET included haemorrhage or secondary degenerative changes (2/7 paragangliomas); siderophages (4/7), mitotic figures (<1-3), capsule (4/7), nested appearance (7/7), confluent nests (6/7), large thin-walled vessels (5/7), cellular pleomorphism (5/7); fibrosis around nests (2/7 focal), haphazard fibrous bands (4/7), and lack of necrosis (7/7). Paragangliomas were chromogranin and synaptophysin positive, but keratin negative. In contrast, the NET were variably positive for chromogranin and synaptophysin, and also keratin positive.

Conclusions: Metastatic paragangliomas can be challenging to diagnose, the principal diagnostic pitfall being metastatic neuroendocrine tumors. Histological clues that can suggest the correct diagnosis include highly vascular tumors composed of cells with foamy cytoplasm and indistinct cell borders. The diagnosis is confirmed by positive staining for chromogranin/synaptophysin but negative staining for keratin.

1663 Various Oncogenic Pathways Are Associated with Distinct Immune and Cancer Gene Signatures in Inflammatory and HNF1a-Inactivated Hepatic Adenomas

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Background: Oncogenic pathways might have a direct influence on the immunological milieu in tumors and expression of oncogenic drivers and immune molecules can impact prognosis and therapy. Hepatocellular adenomas (HCA) are characterized by single genetic abnormalities with constitutive activation of the IL-6 receptor signaling pathway in the inflammatory variant (I-HCA) and inactivation of hepatocyte nuclear factor 1-alpha (HNF1a) in another variant (H-HCA). Therefore, we examined the expression of immune and cancer signature genes in these HCA subtypes.

Design: Tumor mRNA was purified using paraffin-embedded tissue from 5 I-HCA and 6 H-HCA. Transcript abundance of 800 immune signature and 770 cancer signature genes was measured using the NanoString technology. Statistical analysis was performed using NSolver 3.0 companion software.

Results: HCAs differentially expressed 195/800 (24%) immune and 157/770 (20%) cancer signature genes. Unsupervised cluster analysis completely segregated the adenomas by phenotypic subtype according to chemokine, cytokine, complement, TNF superfamily, JAK-STAT pathway, WNT pathway, and chromatin modification gene expression. In addition to CRP and SAA, all I-HCA cases (5/5) exhibited significant upregulation of complement protein C9 and the pleotropic immunomodulatory molecule LIGHT. I-HCA demonstrated increased CCL2 (4/5) and CCL20 (3/5) expression, while H-HCA had increased CCL15 (4/6), CCL16 (4/6), CXCL9 (6/6), and CXCL10 (6/6) expression. H-HCA demonstrated small but significant increases in the inhibitory checkpoint genes FOXP3 (6/6) and LAG3 (6/6). The most significantly upregulated cancer signature genes were phospholipase A2 (PLA2G2A) (5/5) and histone deacetylase 6 (HDAC6) (6/6) for I-HCA and H-HCA, respectively.

HCA Subtype	Immune Regulation Pathway Genes	Oncogenic Pathway Genes
I-HCA	CRP, SAA, C9, LIGHT	PLA2G2A
H-HCA	FOXP3, LAG3, CCL15, CCL16, CXCL9, CXCL10	HDAC6

Conclusions: I-HCA and H-HCA show quantitative differences in immune and cancer signature gene expression and cluster accordingly. Downstream targets of IL6R signaling, as well as complement C9 and LIGHT are highly expressed in I-HCA. Upregulation of HDAC6 in H-HCA suggests that epigenetic dysregulation may have a tumorigenic role in this variant. Chemokine and co-inhibitory molecules are differentially expressed suggesting that distinct oncogenic signaling pathways may influence the immunological microenvironments in different ways.

1664 Reduced Androgen Receptor Expression Supports the Diagnosis of Hepatocellular Carcinoma

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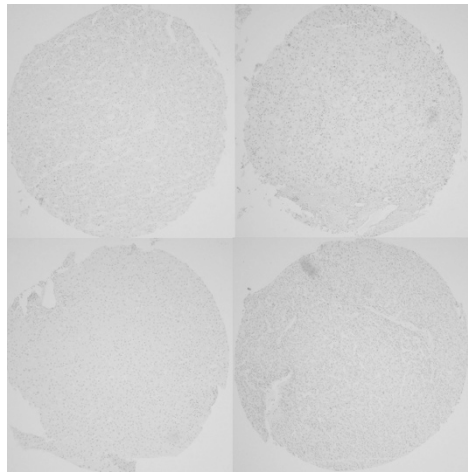
Background: Arginase and Hep-Par 1 immunostains are markers used to confirm hepatic differentiation in hepatocellular carcinoma (HCC); however, their cytoplasmic expression can be weak in poorly differentiated HCC. Additional markers would be valuable in diagnosing HCC. To assess the potential utilization of androgen receptor (AR) as a marker in support of a diagnosis of HCC, we examined whether cells in HCC display altered expression of AR using tissue microarray (TMA).

Design: We collected 82 cases of HCC, including both male and female patients. TMA blocks were constructed with each case represented in triplet, then stained for AR by immunohistochemistry using an autostainer. The nuclear staining of AR was graded from 0 to 3+; the distribution was recorded as focal (<50% positivity) or diffuse (>50% positivity). Additionally, 5 benign liver core biopsies from male and female patients were stained for AR. Prostate tissue was also used as a positive control.

Results: All examined benign biopsies showed 3+ nuclear staining with diffuse positivity in all hepatocytes. In contrast, 78/82 (95%) of HCC cases were negative for AR, and only 3 cases (5%) were positive. Two of the positive cases demonstrated focal 1+ nuclear staining, while the third positive case displayed diffuse 2+ nuclear staining. Prostate tissue was diffusely AR-positive.



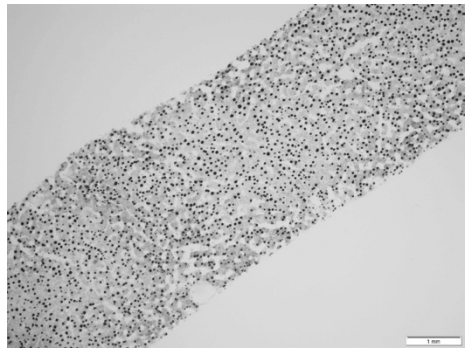


Figure 1	HCC TMA cores with complete negative AR staining
Figure 2	Benign liver biopsy with diffuse 3+ nuclear AR staining

Conclusions: The role of androgens and their receptors in HCC pathogenesis remains incompletely understood, and our observation of expression of AR in HCC by immunohistochemistry has not been previously reported. The absence of AR expression in the majority of examined HCC cases suggests that reduced AR expression may be used as a supportive marker for confirming the diagnosis of HCC. Subsequently, staining for AR in full sections of HCC is necessary to validate our current findings.

1665 The Nearly Normal Liver Biopsy: Presentation, Clinical Associations, and Outcome

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Background: Liver biopsies obtained for abnormal liver enzymes or unexplained ascites occasionally appear histologically nearly normal; in such cases, generating a differential diagnosis can be challenging because there is no published data addressing this topic. This study aims to establish a differential diagnosis and summarize outcomes for nearly normal liver biopsies.

Design: 97 liver biopsies with nearly normal histology were collected from 2 institutions (2002-2006 at institution 1 and 2005-2009 at institution 2). All cases had no significant inflammation, fatty change, biliary tract disease, vascular disease, nodular regenerative hyperplasia, iron overload, metabolic disorder, viral hepatitis, or fibrosis. Minimal changes (<5% steatosis, focal minimal portal inflammation without interface activity, or rare lobular ceroid-laden macrophages) were acceptable. Biopsies for follow-up of known liver disease were excluded. Transplant biopsies, lesion-directed biopsies, biopsies obtained during bariatric surgery, liver donor biopsies, and biopsies for evaluation of methotrexate toxicity were excluded. Individuals were followed by chart review until a clinical diagnosis was made or for a minimum of 1 year.

Results: Biopsy indications are shown in Table1. In 70 patients (72%), a most likely etiology for the clinical abnormality was identified. Etiologies were grouped into categories (Table2). Detailed clinical follow up was available for 66 patients (69%). Liver biochemistries normalized in 32 patients (48.5%) and remained elevated in 34 (51.5%). 7 patients (7.2%) eventually developed chronic liver disease [autoimmune hepatitis (n=3), primary biliary cirrhosis (n=3), and cryptogenic cirrhosis (n=1)].

Biopsy indication (%)	Number (%)
Elevated Liver Biochemistries	77 (79.4)
Portal Hypertension	6 (6.2)
Systemic Illness/Inflammation	5 (5.2)
Varices	4 (4.1)
Elevated Autoimmune Antibodies	4 (4.1)
Hepatosplenomegaly	4 (4.1)
Ascites	3 (3.1)
Cirrhosis by Imaging	2 (2.1)

Etiology	Number (%)
Unknown	27 (27.8)
Systemic Autoimmune Conditions	17 (17.5)
Ischemia/Vascular Insufficiency	13 (13.4)
Metabolic Syndrome	11 (11.3)
Drug	8 (8.2)
GI Inflammation	8 (8.2)
Liver Disease	7 (7.2)
Biliary Outflow Impairment	3 (3.1)
Miscellaneous	3 (3.1)

Conclusions: This multicenter study has determined the differential diagnosis for nearly normal liver biopsies, which will help the pathologists guide subsequent work-up efforts in these histologically and clinically challenging cases.

1666 Liver Tumors in Patients with Familial Adenomatous Polyposis

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Background: Primary hepatocellular neoplasms in patients with familial adenomatous polyposis (FAP) have been reported mostly as isolated case reports. Hence, their prevalence and pathologic features have not been evaluated in detail.

Design: Primary hepatocellular neoplasms were searched from a cohort of 1851 patients with FAP. Detailed histopathologic analysis and reticulin stain was performed on all cases. Immunostains were performed for glutamine synthetase (GS), liver fatty acid binding protein (LFABP), ß-catenin, C-reactive protein (CRP), serum amyloid A (SAA), glypican3 (Gly3) and Ki67.

Results: Eight neoplasms (6 biopsies and 2 excisions) were identified, of which 6 (75%) were hepatic adenomas (3 males and 3 females, mean age 22 years, age range 3 months to 36 years) and 2 (25%) were hepatocellular carcinomas (19 year male and 66 year female). Of the 6 hepatic adenomas, 1 showed mild nuclear atypia, 1 other case showed architectural atypia in the form of focal pseudogland formation, 2 showed abortive portal tracts, 3 showed steatosis and none showed pigmentation or cholestasis. The reticulin framework was intact in all 6 cases. Immunostains were performed on 4 of 6 hepatic adenomas. Based on the immunoprofile, 3 (75%) had an inflammatory phenotype (positive for both CRP and SAA). 2 (50%) cases (including 1 with inflammatory phenotype) showed strong and diffuse GS staining, indicative of ß-catenin activation. LFABP was retained in all cases and none showed nuclear ß-catenin staining or Gly3 positivity. The proliferative index was low in all 4 cases (Ki67 <3%). The hepatocellular carcinomas (1 well differentiated and 1 moderately to poorly differentiated) showed cytologic atypia with loss of reticulin framework. The well differentiated hepatocellular carcinoma was negative for GS and Gly3, with no nuclear ß-catenin expression. The moderately to poorly differentiated hepatocellular carcinoma showed positive staining for Gly3 and GS as well as nuclear ß-catenin staining.

Conclusions: Primary hepatocellular neoplasms are rare in FAP patients (prevalence in our study was 0.004%). Hepatic adenomas are the most common (75%). Activation of the Wnt/ß-catenin pathway appears to play a role only in a subset of the primary hepatocellular neoplasms arising in FAP patients. Hence, other (yet to be characterized) primary oncogenic mechanisms are also responsible for development of primary hepatocellular neoplasms in FAP patients.

1667 Adult Giant Cell Hepatitis: Morphological and Etiological Findings

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Background: Adult giant cell hepatitis (AGCH) is a rare disorder. Its morphological and etiological findings have not been studied in detail.

Design: 15 adult patients with histological diagnosis of giant cell hepatitis in liver biopsies were retrieved from our pathology files between 1996 and 2016. Histologic features including percentage and distribution of giant cell change, inflammation, bile duct injury, cholestasis and fibrosis (by trichrome stain) were evaluated on all cases. Immunostains for cytomegalovirus (CMV), herpes virus (HSV) and varicella zoster virus (VZV) as well as human papilloma virus in-situ hybridization (HPV-ISH) were also performed in available cases. Clinical and laboratory findings were reviewed.

Results: The average age was 52 yrs (range 21 to 75 yrs). 7 were males and 8 females, and 3 (20%) of them were post-liver transplant patients. Mean serum AST was 473 IU/l, ALT was 652 IU/l and alkaline phosphatase was 396 IU/l. Serologies for viral hepatitis B and C were performed in 14 cases and they were all negative. Giant cell change affected on average 40% of hepatocytes (range, 10-90%). In majority of cases (10 of 15, 67%) the giant cell transformation (GCT) was azonal, while 20% (3 of 15) cases showed GCT in zone 1 region and 13% (2 of 15 cases) showed GCT in the zone 3 region. Portal inflammation was absent to mild in 73% (11/15) while lobular inflammation was absent to mild in 60% (9/15) of cases. None of the cases showed a prominent plasma cell infiltrate. Hepatocanalicular cholestasis was common (53%, 8/15 cases). Bile ducts were intact in all 15 cases and 27% (4/15) of cases showed bile ductular proliferation. 47% (7/15) of cases showed bridging fibrosis or cirrhosis while 33% (5/15) of cases showed pericellular fibrosis. HPV-ISH as well as immunostains for CMV, HSV and VZV were performed in 9 cases and they all were negative. Subsequent clinical workup identified the following etiologies: autoimmune hepatitis (AIH) - 53% (8/15), idiopathic - 40% (6/15) and herbal drugs - 7% (1/15). 10 follow-up biopsies on 6 patients were available and all continued to have giant cell change. Clinical follow-up was available in 14 patients (range 3 weeks to 15 years) and 7 had died (3 of liver failure and 4 unrelated to liver disease).

Conclusions: In our series AIH (53%) followed by idiopathic (40%) were the two most common associations with AGCH. Viral etiology is unlikely to be a cause of AGCH. The histological findings did not readily distinguish between the various etiologies. None of the cases associated with AIH showed a plasma cell rich infiltrate or other typical morphological findings of AIH.

1668 Brown Stains for Identification of Ballooned Hepatocytes in Non-Alcoholic Steatohepatitis (NASH): Ready for Prime Time?

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Background: The presence of ballooned hepatocytes, a manifestation of cellular damage, is required to establish a diagnosis of NASH and distinguish it from simple steatosis. Intra- and interobserver variability for identification of ballooned hepatocytes on hematoxylin-eosin stain is high. Immunohistochemistry (IHC) for ubiquitin and keratins (K) 8/18 have been proposed for improving detection of ballooned hepatocytes.

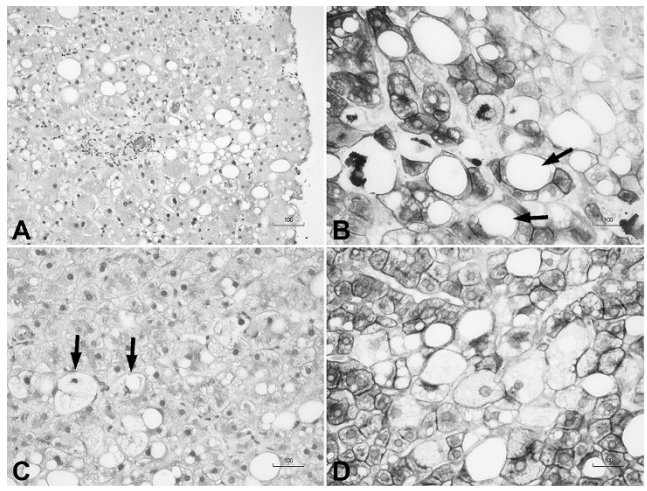
Design: We identified 37 consecutive biopsies by a text search for "liver" and "steatosis". IHC for ubiquitin and K8/18 was performed on all cases. Each biopsy was graded for steatosis, ballooning and inflammation by already established NASH activity score (NAS). IHC stained slides were graded for ballooned hepatocytes as 0 (absent), 1 (few ballooned cells) and 2 (many ballooned cells). Ballooned hepatocytes were identified by loss of cytoplasmic staining, which is present diffusely in normal hepatocytes, with or without aggregation in Mallory Denk bodies.

Results: Agreement between 2 pathologists for identification of ballooned hepatocytes on HE and IHC

HE	Ubiquitin	K8/18
0.480	0.491	0.624

Agreement between HE and IHC for identification of ballooned hepatocytes

Ubiquitin	K8/18
0.395	0.527



Ballooned hepatocytes were easily identified when there was staining of Mallory Denk bodies (A,B) or when there was absence of staining (C, D). However, interpretation is impossible without a morphological context. For eg, lipid vacoules (B, arrows) do not stain but are not interpreted as ballooned hepatocytes due to their morphological shape. A lobular gradient of staining for K8/18 makes it difficult to identify true ballooned hepatocytes (B, right upper corner).

Conclusions: IHC for ubiquitin and K8/18 easily identify classic ballooned cells with well formed Mallory Denk bodies. These are however also easily identified on HE stain. IHC is only marginally useful in identifying ballooned cells that do not contain Mallory hyaline or that are few in number.

IHC for K8/18, but not ubiquitin marginally improves inter-observer agreement in identification of ballooned hepatocytes; accurate interpretation requires proper morphological context.

1669 Hepatic Sclerosed Hemangioma Can Mimic Segmental Atrophy

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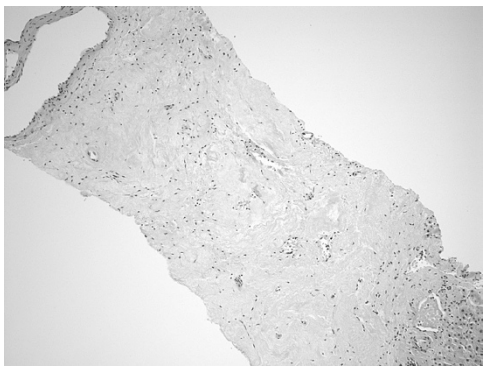
Background: Segmental atrophy (SA) of the liver is a recently described pseudotumor with a broad differential diagnosis. Hemangioma is the most common benign neoplasm of the liver and can display a range of histologic features. We have observed that sclerosed hemangioma (SH) can masquerade as the nodular elastosis stage of SA, particularly when ectatic vasculature is not evident.

Design: For this study, we identified 25 cases in our departmental archives: 19 diagnosed as SH, five diagnosed as SA, and one signed out descriptively. Several morphologic characteristics, including vascular component and properties of background matrix material, were examined on H&E-stained sections. Elastin and CD34 staining was performed on available tissue.

Results: Nineteen cases were established as SH and six as SA (one SA was originally diagnosed as SH, and one vice versa; one SA was signed out descriptively). Seven of these cases resembled both diagnoses, necessitating a judgment call. Patients with SA were older than those with SH (median 69 years vs. 61 years, P=0.109) and were more often female (83% vs. 63%, P=0.624). Both SA and SH demonstrated predominately pale eosinophilic to amphophilic amorphous matrix material, with scant cellularity and variably fine and thick fibers. SH sometimes showed diffuse thin ectatic/anastomosis vascular channels and other times sclerotic regions with rare vascular channels, mimicking SA (Fig. 1). All SA contained scattered vessels, typically present at tumor edge and highlighted by CD34. SH more commonly demonstrated matrix edema (58% vs. 17%, P=0.16), while SA more commonly showed dense or diffuse elastin staining (42% vs. 100%, P=0.041), ductular proliferation (6% vs. 50%, P=0.031), and thick-walled blood vessels (42% vs. 83%, P=0.16). Vascular thrombi were seen at the same frequency in both diagnoses (P=1.0).



Conclusions: The distinction between SA and SH can be challenging, depending on sampling. As SA has only been recently described in the literature, SA may erroniously be diagnosed as SH. Matrix edema may indicate SH, while ductular reaction and strong matrix elastin staining can favor SA. CD34 staining is less helpful, as both lesions are well vascularized. While these entities are considered benign, diagnostic accuracy is critical for further understanding of SA.

1670 Characterization of Morphology, Microsatellite Instability, and Tumor Immune Microenvironment in Intrahepatic Cholangiocarcinoma

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Background: Intrahepatic cholangiocarcinoma (ICC) is an aggressive disease that can be divided into at least two morphological subclassifications with distinct immunohistochemical profiles and genetic aberrations. The success of immune checkpoint blocking antibodies as adjuvant therapy in other malignancies has raised the possibility that similar mechanisms may occur in ICC. The purpose of this study is to further characterize the relationship between tumor morphology, microsatellite instability status (MSI), and immune markers in ICC.

Design: From 2001 to 2015, there were 21 resections for ICC at our institution. Clinicopathologic data were collected for all cases. Slides were reviewed for histologic classification, and MSI testing was performed. IHC was used to evaluate S100P and PD-L1 expression by the tumor cells and to characterize the nature of the host inflammatory response. Tumor infiltrating lymphocytes (TILs) were graded in both the intraepithelial compartment and the tumor stroma as 0 (none), 1 (<5%, focal), 2 (5-25%, moderate), and 3 (>25%, diffuse). The intensity of inflammation at the invasive tumor front was also graded from 0 (none) to 4 (dense continuous band).

Results: In this cohort the average patient age at presentation was 63, and 67% of patients were women. Three patients underwent tumor embolization prior to resection, but none were treated with neoadjuvant chemotherapy. The median follow-up was 15 months. Using newly defined morphologic criteria, 8 (38%) of the ICC resection specimens were bile duct/type 1 ICCs(positive S100P), and the remaining 13 (62%) were cholangiolar/type 2 ICC. No ICC in our cohort exhibited microsatellite instability. Only 2 (10%) cases (one type 1; one type 2) demonstrated strong, membranous PD-L1 immunoreactivity within tumor cells, while 17(81%) of the ICCs (six type 1; eleven type 2) had total absence of PD-L1 expression. These two cases also demonstrated frequent to abundant PD-L1 staining of lymphocytes at the tumor interface, as well as a moderate grade 2 CD8+ TILs, but this association was not significant when compared to the PD-L1 negative tumors.

Conclusions: We demonstrate that ICCs may rarely express PD-L1 in tumor cells and in lymphocytes at the invasive front. These data suggest that a minority of ICC have adaptive immune resistance that may respond to anti-PD1/PD-L1 therapy. Morphologic subtype does not appear to correlate with immune response, and MSI was not identified; neither can be used as a surrogate for immune tolerance in this tumor type.

1671 A Unique Group of Fibrolamellar Carcinomas Lack the Classic *DNAJB1-PRKACA* Fusion and Instead Have PRKAR1A Loss

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Background: Fibrolamellar carcinomas are characterized by activation of Protein kinase A, a kinase composed of catalytic and regulatory subunits. Almost all fibrolamellar carcinomas have a heterozygous 400kb deletion that leads to the fusion of *DNAJB1* and *PRKACA*. *PRKACA* encodes a catalytic subunit of Protein kinase A and the *DNAJB1-PRKACA* fusion transcript activates the Protein kinase A pathway. Protein kinase A also has a regulatory subunit encoded by *PRKAR1A* and we hypothesized that loss of function of the regulatory unit could also lead to Protein kinase A activation and thus to fibrolamellar carcinoma. Since *PRKAR1A* mutations underlie the Carney complex, we searched for liver tumors in individuals with the Carney complex.

Design: We reviewed the histology of liver tumors that arose in patients with a personal or family history of the Carney complex, along with 5 control sporadic fibrolamellar carcinoma, all of which have the *DNAJB1-PRKACA* fusion gene. On all cases, immunostains were performed for CK7, CD68, Arginase, and PRKAR1A. FISH for *PRKACA* rearrangement was performed on all cases and controls. *PRKAR1A* was sequenced in two tumors.

Results: We identified 4 individuals (2 men and 2 women; ages 7, 14, 53 and 68 years) with liver tumors and a personal history of the Carney complex. All 4 cases displayed the typical morphology of fibrolamellar carcinoma and were positive for Arginase, CK7 and CD68. FISH was negative for *PRKACA* rearrangement, showing intact *PRKACA* loci with normal copy numbers. However, *PRKAR1A* sequencing in 2 cases identified heterozygous pathogenic mutations; one in each case (p.W190*; p.R96*). In addition, all 4 cases were negative for PRKAR1A by immunohistochemistry, consistent with inactivation of this key regulatory unit of Protein kinase A. As expected, the 5 controls were all positive for CK7, CD68 and Arginase expression and *PRKACA* rearrangement by FISH.

Conclusions: Fibrolamellar carcinoma can arise in individuals with the Carney complex and is characterized by loss of PRKAR1A expression, inactivating *PRKAR1A* mutations and the absence of the *DNAJB1-PRKACA* fusion gene. *PRKAR1A* mutations provide an alternate means for activation of Protein kinase A. Patients with the Carney complex and their relatives should be considered for screening for fibrolamellar carcinoma.

1672 Immunohistochemistry (IHC) as a Valuable Aid in the Detection of Early Oxaliplatin-Associated Liver Injury

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Background: Liver metastases from colorectal cancer is a common complication which can be treated by surgery. With the routine use of the neoadjuvant drug oxaliplatin, the prognosis after such surgery has vastly improved. However, increasing use of oxaliplatin has been associated with varying degrees of liver injury which can compromise the benefits of surgical therapy, rarely even leading to death.

Design: Liver tissue from 54 patients who had undergone hepatectomy after taking oxaliplatin were initially chosen. All cases with non-tumor liver tissue within 2 cm of the tumor area or with significant steatosis were excluded from the study, resulting in 50 cases. Liver tissue from 6 cases of benign liver tumor (either adenoma or focal nodular hyperplasia) were selected as negative controls. Histologic findings known to be associated with oxaliplatin-associated liver injury (OALI) were studied using H&E and trichrome sections: H&E for sinusoidal dilatation, peliosis, nodularity and steatosis, and trichrome for perisinusoidal and centrilobular fibrosis. H&E slides were graded from 0 to 3, and trichrome slides from 0 to 2. Histologic scores were calculated by summing up the H&E and trichrome grades for cases and controls. The liver sections were stained with CD34 (to detect sinusoidal capillarization), smooth muscle actin or SMA (to detect hepatic stellate cell activity) and glutamine synthase or GS (to detect aberrant expression in non-centrizonal hepatocytes), to look for OALI. The IHC sections were then graded from 0 to 3, and the individual grades for each stain were added to form an IHC score. Students paired T-test was used for statistical calculations.

Results: Average histologic score for the cases was 4.7 and for controls was 1.83. Average IHC scores for cases was 5.36, and that for controls was 3.1. Positive staining for CD34, SMA and GS were 96%, 94% and 98% respectively in the patient population; number of patients with 1, 2 and 3 scores for CD34 were 13 (26%), 26 (52%) and 9 (18%), for SMA were 12 (24%), 24 (48%) and 11 (22%), and for GS were 17 (34%), 30 (60%) and 2 (4%). Statistically, staining for CD34 and GS were found to be significantly more frequent in patients than in controls, with p-values of 0.03 and 1.4×10^{-6} respectively. SMA did not reach statistical significance (p-value 0.47). The average IHC score for cases however did reach statistical significance (p-value 0.0003).

Conclusions: Judicious use of liver biopsy and immunohistochemical markers like CD34 and GS can be very useful to diagnose OALI early, and prevent irreversible liver damage.

1673 Glutamine Synthetase Immunostaining in Regressed Cirrhosis

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Background: The prevailing view that cirrhosis is irreversible has been challenged. It is now accepted that varying degrees of regression can be achieved if the agent of injury is removed. The advent of drugs that cure hepatitis C (HCV) has also made regression a leading topic of clinical interest; it can potentially obviate long-term hepatocellular carcinoma surveillance. Therefore it is important for pathologists to recognize regressed cirrhosis (RC). In the normal liver, glutamine synthetase immunostaining (GS) is expressed around central veins (CV). In RC, although fibrous bands between portal

tracts (PT) & CV may largely be resorbed, the abnormal PT-CV adherence often remains. Hence we hypothesized that aberrant GS positivity *adjacent to PT* (PT-GS+) would help recognize RC.
Design: We performed GS on 52 livers (26 explants, 9 biopsies, 17 resections), including 14 RC, 15 normal, 5 nodular regenerative hyperplasia (NRH), and 18 cirrhotic livers. Etiologies of liver disease in RC and cirrhotic groups included 4 cryptogenic, 17 HCV, 2 AIH, 2 HBV, 2 NASH, and 5 ETOH. Qualification for RC required histologic features as previously described: curved delicate incomplete septa, PT-CV adhesions, and PT "remnants" (PTs with no venous branch). 12/14 RC had baseline cirrhosis established based on previous biopsy (9) or signs of cirrhosis based on physical exam, laboratory & radiological findings. In the RC group, 3 had HCV treatment with viral eradication, 1 had HBV treatment, and 2 ETOH cirrhosis pts stopped drinking ETOH.
Results: The average age was 57 (M=31, F=21). All RC (100%) had areas of aberrant PT-GS+, indicating that PT-CV approximation had occurred ($p<0.0003$ compared to all other categories). No normal cases had PT-GS+, but all had the expected CV-GS+. Half of cirrhosis cases had areas showing regression, including PT-GS+. Cirrhotic cases that did not show regression had almost complete loss of GS or only focal perivenular GS, as previously reported. Only 1 NRH showed PT-GS+. Overall, PT GS+ showed highly significant differences between the 4 categories ($p<0.00001$).
Conclusions: This study shows that aberrant PT-GS+ is present in RC and can be useful in identifying regression. It validates previous literature that areas of regression are found in many cirrhotic livers. It also underscores the inadequacy of current staging systems to indicate RC, and affirm clinicians' high suspicion for cirrhosis that have then histologically regressed. In conclusion, pathologists can use GS to help diagnose RC.

1674 Multifocal Hepatocellular Carcinoma with Features Suggestive of Intrahepatic Metastases: Clinical, Histological and Radiological Correlation

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Background: Limited studies attempted to distinguish two possible subtypes of multifocal hepatocellular carcinoma (HCC), i.e., multicentric origin versus intrahepatic metastases (IM) of a primary HCC. Recent studies suggest that HCC presented with a dominant mass and multiple satellite nodules is more likely of IM type, and is associated with poor prognosis. We sought to study clinical, pathology, radiology findings in these patients to identify potential clues to the pathogenesis of multifocal HCC of IM type.
Design: Multifocal HCC with a dominant mass and at least 5 additional satellite nodules in resection specimens without any prior anti-tumoral therapy were retrieved from our institute from 2000-2016. The slides were reviewed for histologic characteristics and medical charts were reviewed for clinicodemographics and radiological findings. Survival data were compared with SEER cancer database.
Results: Thirteen cases were included (11 male and 2 female patients; age of HCC diagnosis: 46-77 years old). Ten cases had chronic viral hepatitis as underlying liver disease and 10 (of 13, 77%) occurred in non-cirrhotic liver. All cases showed multifocal HCC with a dominant mass (size range 2.0-20.0 cm) and many satellite nodules (size range 0.1-3.5 cm), with the farthest satellite nodule being 9.0 cm away from dominant one. Twelve cases were moderately differentiated HCC and 1 was poorly differentiated. Tumor heterogeneity was seen in 4 cases, in which the satellite lesions showing similar or higher tumor grade. Pathology staging included T2 (5 cases), T3a (4 cases), T3b (3 cases) and T4 (1 case). Small vessel invasion was identified in 11 cases, in which 3 cases also had large vessel invasion. While majority of cases had tumor invasion of portal veins, one case showed both sinusoidal and portal vein invasion. While many satellite HCC nodules were identified histologically, preoperative imaging studies failed to detect those satellite nodules in 8 (of 13, 62%) cases. The 2-year survival rate was 20% in 10 cases with at least 2 years of follow-up, and 25% in 4 cases with pathological staging of T2, in comparison to 55.8% in localized HCC patients from SEER database.
Conclusions: The results of this pilot study suggest that multifocal HCC of IM type acts an aggressive behavior, which may not be truly reflected by AJCC tumor staging system, especially in those staged as mpT2. Many of those cases are understaged by preoperative imaging studies. IM of HCC occurs mostly through portal vein invasion, but also through sinusoidal spreading.

1675 Identifying Mutation Profile in Hepatocellular Carcinoma by Targeted Next-Generation Sequencing

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Background: Although significant progress has been made to uncover genetic alterations in hepatocellular carcinoma (HCC), our understanding of genetics involved in the initiation and progression of HCC is far from complete. Recent studies have attempted to subclassify HCC into two groups, in which aggressive group showed activation of MET, TGF-beta, or caonical Wnt pathway, or had TP53 mutation, while less aggressive group showed CTNNB1 mutation or activation of liver specific Wnt pathway. We sought to study mutation profile in HCC using targeted next-generation sequencing (NGS).
Design: Primary HCC in resection specimens without any prior anti-tumoral therapy were retrieved from our institute from 2000-2006. DNA was isolated from formalin-fixed, paraffin-embedded blocks, followed by target enrichment using IDT hybridization probes covering 76 genes then sequencing on Illumina's NextSeq 500 sequencer. For variant calling Basespace Onsite with BWA Enrichment v2.1 analysis was used. Mutation status was correlated with clinicopathologic variables.
Results: Thirty cases were included. Overall, mutations were identified in 28 genes out of the 76-gene panel. Majority of HCC harbored multiple gene mutations ranging from 2 to 15 mutations. Most common mutated genes identified in our study were MET (23%), PDGFRA (20%), EZH2 (17%), TP53 (13%), CTNNB1 (13%), and myc (6.7%). No significant correlation was identified between the mutation status and clinicodemographics, underlying liver diseases (virus hepatitis, alcohol and others), or histological findings (presence or absence of cirrhosis in the background liver, tumor pathologic grading and staging). In addition, there was no correlation of MET mutation status with MET expression level determined by immunohistochemical studies.
Conclusions: By targeted NGS, we identified common gene mutations in HCC including MET, PDGFRA, EZH2, TP53 and CTNNB1. No significant correlation of mutation status with clinicopathologic findings was seen which might be due to limited sample size. No correlation of MET mutation status with MET expression level was seen.

1676 Atypical Hepatocellular Neoplasms: Mutational Profile and Copy Number Alterations

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Background: Tumors with borderline features between adenoma and hepatocellular carcinoma (HCC) have been termed as 'atypical hepatocellular neoplasms' (AHN) or 'hepatocellular neoplasms with uncertain malignant potential'. These tumors may have β-catenin activation and/or focal cytoarchitectural atypia/reticulin loss that is insufficient for HCC diagnosis. This study examines the mutational profile and copy number alterations in AHN.
Design: Capture-based next generation sequencing (NGS) targeting the coding regions of 510 cancer genes and select introns, covering a total of 2.8 Megabases (MB) was performed in 12 AHNs. Somatic single nucleotide variants (SNV), insertions/ deletions (indels), and copy number alterations (CNA) were evaluated.
Results: Diffuse (≥50%) glutamine synthetase (GS) was seen in 9 AHN (8 diffuse homogeneous, 1 diffuse heterogeneous); other 3 AHN did not show diffuse GS. Pathogenic mutations in *Wnt*-signaling pathway genes were identified in all 9 AHN with diffuse GS: *CTNNB1* exon 3 mutation (3 cases) or deletion (4 cases), *CTNNB1* exon 7 mutation (1 case), *APC* mutation (1 case), *AXIN1* mutation (1 case). The case with diffuse heterogeneous GS had both *AXIN1* and *CTNNB1* exon 7 mutation. Additional pathogenic drivers were present in 3/9 (33%) cases and included mutations in *IDH1, KEAP1, SYNE1,* or *WT1*. CNA typically seen in HCC (8p-,8q+,16+) were present in 3/9 (33%) AHN with diffuse GS. Of the 3 cases without diffuse GS, none had CNA, and 2 did not have pathogenic mutations; 1 case had mutations in *HNF1A*, *NOTCH1*, and *ERBB4*. None of the 12 AHN had *TERT* promoter mutations.

Category	Mutations	Copy number changes
AHN, diffuse GS (n=9)	CTNNB1 (exon 3): 7 cases.CTNNB1 (exon 7): 1 case. KEAP1: 1 case. APC, WT1 and SYNE1: 1 case. AXIN1: 1 case. IDH1ï»¿: 1 case	3/9 cases: 8p- (2 cases), 8q+ (1 case)
AHN, without diffuse GS (n=3)	HNF1A, ERBB4, NOTCH1: 1 case	None

Conclusions: Genetic basis for diffuse GS was evident in all 9 AHN with diffuse GS; mutations in components of *Wnt*-signaling pathway other than *CTNNB1* exon 3 (*APC*, *AXIN1*) may explain β-catenin activation in a subset of cases with diffuse GS. AHN frequently show mutations involving genes that have been reported in HCC, including *KEAP1, SYNE1, AXIN1*, and *NOTCH1*. CNA that have been described in HCC such as 8p-, 8q+, 16+ are seen in one third of cases with diffuse GS, but not in cases without diffuse GS. Mutational profile and/or CNA may be helpful in risk stratification of AHNs.

1677 Cancer Gene Sequencing of Hepatic Small Vessel Neoplasm (HSVN) and Hepatic Cavernous Hemangioma

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Background: Hepatic small vessel neoplasm (HSVN) is a recently described infiltrative hepatic vasoformative neoplasm composed of small vessels without diagnostic features of cavernous hemangioma or hepatic angiosarcoma. Somatic activating mutations have been recently reported in *GNAQ* and *GNA11* in association with cutaneous congenital hemangiomas, which share similar morphologic features with HSVN. In our initial HSVN series we identified hotspot mutation in *GNAQ* in 2 HSVNs, but not in cavernous hemangiomas (CH), which raises the possibility that HSVN and congenital hemangioma may share a similar mechanism of disease. We also reported on hepatic vascular neoplasms with features of both HSVN and CH, which we termed variant CH (vCH), which share similarities with hepatic anastomosing hemangioma.
Design: DNA was extracted from an expanded cohort including 9 HSVN, 7 vCH, and 6 classic CH (with matched normal tissue). Next generation sequencing was performed targeting exons of 510 cancer genes and select introns from 40 genes. Duplicate reads were removed computationally for accurate determination of allele frequency and copy number alterations. Single nucleotide variants, insertions/deletions, copy number alterations, and rearrangements were evaluated.
Results: All HSVN had simple genomic profiles with no copy number changes and low mutation burden.

Cases	Mutations	Copy Number Alterations
HSVN (n=9)	GNAQ p.Q209H (2/9 cases)PIK3CA p.H1047R (1/9 cases)	None
vCH (n=7)	GNAQ p.Q209H (1/7 cases)PIK3CA p.G118D (1/7 cases)PDGFRB p.K653E (1/7 cases)	Losses of 3, 9, 13, 15, 16, 17 and gains of 7, 12, 18 (1 case)
Classic CH (n=6)	None	None

Conclusions: In contrast to classic CH, a minor subset of both HSVN and vCH have hotspot mutations in *GNAQ* (p.Q209H), which is the same pathogenic mutation reported in association with cutaneous congenital hemangiomas, suggesting a similar underlying biology. While the presence of a few additional hotspot mutations and chromosomal gains and losses in 3 of 16 HSVN or vCH, raise concern for possible malignant potential, the majority of HSVN and vCH lack pathogenic mutations. Thus, despite an infiltrative growth pattern in HSVN, the molecular profile argues for consideration as benign vascular neoplasms, which is consistent with our follow up data.

1678 In Situ Hybridization for MiR-21 in Hepatocellular Carcinoma

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Background: Micro-RNAs (miR) are short untranslated RNA transcripts involved in the regulation of gene expression. As miR are untranslated, they cannot be investigated by immunohistochemistry. MiR-21 has been shown to promote carcinogenesis through down-regulation of tumor suppressors, particularly PTEN. Molecular testing has shown that mir-21 is upregulated in hepatocellular carcinoma (HCC). The aim of this study is to determine if miR in-situ hybridization (ISH) is feasible on formalin fixed paraffin embedded (FFPE) liver tissue on an automated platform, and specifically if miR-21 ISH is useful for distinguishing HCC from benign hepatocytes. If so, this test could have utility, as currently available ancillary tests have limited sensitivity and specificity.
Design: MiR-21 ISH was performed using the ViewRNA eZ-L Kit on Leica Bond III platform on FFPE tissue microarrays containing 48 HCCs with matched non-tumor tissue. Positive (Rnu6) and negative (dapB) controls were used. Results were quantified based on a composite score comprising the percentage of cells with signal and the number of signals per cell, as follows: 0: no signal, 1: 1-33% of cells with signal, 2: 34-66% of cells with signal, 3: 67-100% of cells with signal; 0: <3 signals/cell, 1: 3-5 signals/cell, 2: 5-10 signals/cell, 3: >10 signals/cell. Cases were considered miR-21 positive if the composite sum was 2 or more.
Results: 14 tumor cases and 10 benign controls were excluded due to tissue loss or negative Rnu6 staining (mainly older cases). Of the remaining 34 HCCs, 32 showed positivity for miR-21, whereas no matched benign tissue achieved a positive score (sensitivity 94%, specificity 100%, $p<0.0001$). Additionally, higher miR-21 score correlated with worse tumor differentiation (high-grade mean score=3.67 vs. low-grade mean score=2.86), with results approaching statistical significance ($p=0.0690$).
Conclusions: MiR-21 ISH can distinguish benign from malignant liver cells with high sensitivity and specificity and may be correlated with differentiation. The ability to test for miRs in FFPE tissue by ISH on an automated platform may have significant diagnostic, prognostic, and investigational implications.

1679 In-Situ Hybridization for Glypican-3 RNA Is More Sensitive Than Immunohistochemistry in Hepatocellular Carcinoma

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Background: Glypican-3 (GPC3), a cell surface oncofetal proteoglycan, is a useful marker for hepatocellular carcinoma (HCC) since it generally does not mark benign hepatocytes and rarely reacts with non-hepatic tumors by immunohistochemistry (IHC). However, IHC is hampered by low sensitivity and the degree of expression can only be graded in a rudimentary fashion. We evaluated RNA in-situ hybridization (ISH) for GPC3 in HCC.
Design: 127 HCCs from 2 institutions (all formalin fixed paraffin embedded (FFPE)) were evaluated for GPC3 by ISH and IHC. RNA ISH for GPC3 was performed using the automated ViewRNA eZ-L kit (Affymetrix, Santa Clara, CA) which is based on branched DNA technology. The ISH results were quantified based on the percentage of cells with signal and the number of signals per cell, as follows: 0: no signal, 1: 1-33% of cells with signal, 2: 34-66% of cells with signal, 3: 67-100% of cells with signal; 0: <3 signals/cell, 1: 3-5 signals/cell, 2: 6-10 signals/cell, 3: >10 signals/cell. IHC was performed by standard protocol with a commercially available antibody and graded as positive/negative.
Results: 116 of 127 HCCs showed hybridization to GPC3 RNA for a sensitivity of 91% compared to IHC, where 92 of 127 HCCs were positive for a sensitivity of 72%. Thus, ISH is more sensitive than IHC ($p=.0001$). Results were concordant in 98 of 127 cases (77%). For ISH, the median score for percentage of cells staining was 2 (34-66% of cells with signal) and the median score for signals/cell was 3 (>10). 4 ISH negative cases were IHC positive and 25 IHC negative cases were ISH positive.
Conclusions: GPC3 RNA expression can be detected in FFPE by ISH and is more sensitive than IHC. It further allows for quantification. Additional studies will be needed to determine the specificity of the ISH platform.

1680 Albumin In-Situ Hybridization May Be Positive in Adenocarcinomas and Other Tumors from Diverse Sites

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Background: Albumin in-situ hybridization (ISH) is a marker of hepatocellular differentiation used to aid in the diagnosis of hepatocellular neoplasms. Positive albumin ISH has been observed in intrahepatic cholangiocarcinoma (CCA) and some pancreatic acinar cell carcinomas (pACC) but is otherwise reported as specific for hepatocellular differentiation. Prior studies have been limited by the use of tissue microarrays and use of only one of 2 commercial vendors.
Design: Whole slide sections were reviewed from 209 clinically and radiologically characterized tumors, including hepatocellular carcinomas (HCC), various adenocarcinomas, renal cell carcinomas, pACC and yolk sac tumors (see table). Albumin ISH (Advanced Cell Diagnostics, ACD) was performed on all specimens and 38 cases were co-tested with another commercial kit (Affymetrix, AFX). ISH was considered positive if >5% of tumor cells stained. Immunohistochemistry was performed for Arginase in cases positive for albumin ISH.
Results: Positive albumin ISH was observed in all HCC, most intrahepatic CCA and a subset of pACC, breast, gallbladder, lung, and hepatoid adenocarcinomas (Table). The 2 kits were concordant in 36 of 38 specimens:27 of the 29 ACD-positive cases were also positive by AFX (except 1 intrahepatic CCA and 1 gallbladder carcinoma)and 9 cases were negative with both ACD and AFX kits. The AFX kit yielded less intense staining than the ACD kit in all cases. Arginase was expressed in 3 intrahepatic CCA and 1 hepatoid adenocarcinoma but was negative in all other non-HCC.

Diagnosis	Cases (n)	Positive, n(%), ACD
Intrahepatic CCA	27	22 (81.5)
HCC	26	26 (100)
Renal cell	17	0 (0)
Gastric	16	0 (0)
Ampullary	16	0 (0)
Colorectal	15	0 (0)
Extrahepatic CCA	14	0 (0)
Lung	13	3 (23.1)
Gallbladder	13	5 (38.5)
Pancreatic	13	0 (0)
Adrenal	11	0 (0)
Breast	11	1 (9.1)
Yolk sac	8	2 (25)
Acinar	6	2 (33.3)
Hepatoid	3	1 (33.3)

Conclusions: Albumin ISH is a very sensitive marker of hepatocellular differentiation, but is not perfectly specific. It is often positive in intrahepatic CCA and may be falsely positive in carcinomas from several sites. The almost complete concordance of all cases tested by both vendors indicates similar assay performance. It is important to understand these limitations when interpreting albumin ISH, especially in small biopsies.

1681 Clinicopathologic Features of Hepatic Lymphoma, a Large Retrospective Study

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Background: Although the liver is commonly involved by systemic lymphoma, primary hepatic lymphoma (PHL) is rare with only a few published small case series.
Design: We identified 202 cases from 191 patients who had a tissue diagnosis of hepatic lymphoma at UCSF Medical Center between 1982 and 2016.
Results: We identified 117 surgical pathology (biopsy/resection) cases, 53 autopsy cases, and 32 fine needle aspiration cases that included 56 cases of primary hepatic lymphoma (PHL), from 51 patients, and 146 cases of secondary hepatic involvement by systemic lymphoma (SHL), from 140 patients. In this study, the most common types of lymphoma identified in liver were post-transplant lymphoproliferative disorder (PTLD, n=47), diffuse large B cell lymphoma (DLBCL, n=45), classical Hodgkin lymphoma (CHL, n=16), and NK/T cell lymphoma (NK/T, n=11). The most common types of PHL were PTLD (n=23), DLBCL (n=15), and Burkitt lymphoma (BL, n=3). Mean age was similar (PHL, age 56; SHL, age 58) with male predilection in both groups (M:F=2:1 in PHL and 2.5:1 in SHL). 78% of PHL patients presented with discrete liver lesion, which was more common than in SHL (54%). Of interest, the majority of autopsy cases showed diffuse infiltration with no discrete liver lesion and none of them demonstrated a solitary liver nodule and thus, disease may not have been apparent on imaging. At the time of biopsy/resection, SHL patients presenting with a solitary liver lesion had a favorable prognosis compared to SHL without discrete liver lesions (average follow up time: 48 months for solitary lesions; 28 months for multiple lesions; 10 months for cases with no discrete lesions, $p<0.05$). In particular, primary hepatic DLBCL had a significantly longer follow up time compared to systemic DLBCL (68 months for primary versus 8 months for systemic, $p<0.01$). 82% of PTLDs in liver recipients showed EBV positivity, which was more common than in patients with other organ transplants (55%). 78% of PTLDs in liver recipients presented as PHL. In comparison, only 40% of PTLDs in patients of other organ transplants presented as PHL.
Conclusions: In our experience, PTLD and DLBCL are the most common subtypes of primary hepatic lymphoma and, among systemic lymphomas, CHL and NK/T-cell lymphoma were also common (compared to only a single case of hepatosplenic T cell lymphoma). There is no significant difference in age or gender, but primary hepatic DLBCL may have a better prognosis than DLBCL arising in other sites and the underlying mechanism of disease could be further investigated.

1682 Detection of Albumin Expression by RNA *In Situ* Hybridization Is a Sensitive and Specific Method for Identification of Hepatocellular Carcinomas and Intrahepatic Cholangiocarcinomas

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Background: Recent studies suggested that detection of albumin expression by RNA *in situ* hybridization is a highly sensitive method for identification of hepatocellular carcinomas (HCCs) and intrahepatic cholangiocarcinomas (ICCs)/hepatoid carcinomas (HCs), with a diagnostic sensitivity of nearly 100% for HCCs and a range of 70-100% for ICCs/HCs. We investigated the diagnostic sensitivity and specificity of RNAscope® (ACD) in the detection of albumin expression in HCCs, ICCs, and carcinomas from various organs.

Design: RNA *in situ* hybridization (RNAscope) for albumin was performed on 482 cases on tissue microarray sections, including HCCs (N=37), breast ductal carcinomas (CAs) (N=68), lung adenocarcinomas (ADCs) (N=40), esophageal ADCs (N=35), pancreatic ADCs (N=50), urothelial CAs (N=40), endometrial ADCs (N=40), endocervical ADCs (N=26), ovarian serous CAs (N=42), papillary RCC (N=32), mesotheliomas (N=22), and papillary thyroid CAs (N=50). RNAscope for albumin was also performed on 22 cases of ICC, including 14 surgical resection specimens and 8 needle core biopsy specimens. The targeted probes prepared by ACD covered the albumin sequence from nucleotides 2 to 490. The scoring system for RNAscope was based on these criteria: 0 (0 or 1 dot/tumor cell), 1+ (2-3 dots/tumor cell), 2+ (4-10 dots/tumor cell), 3+ (>10 dots/tumor cell), and 4+ (>10 dots/tumor cell with clusters of signal).

Results: The results of RNAscope for albumin showed that 36 of 37 (97%) HCCs had detectable mRNA, with diffuse staining (3+ or 4+) in all cases, whereas only 1 lung ADC showed 3+ staining for albumin, and the remaining non-HCC and non-ICC cases were negative for albumin. The lung ADC case with expression of albumin was also positive for TTF1 and had no hepatoid histomorphology. Fourteen of 22 ICCs (64%) were positive for albumin, with diffuse staining in 10 cases. Among these 22 ICCs, 10 of 14 (71%) resection specimens and 4 of 8 (50%) needle core biopsy specimens were positive for albumin.

Conclusions: This data demonstrates 1) that RNAscope for albumin is a highly sensitive and specific method for identifying HCCs; 2) RNAscope for albumin is a highly specific and moderately sensitive method for detection of ICCs; 3) a large sample size may increase diagnostic sensitivity; and 4) caution should be taken because rare carcinomas (non-HCC, non-ICC, and no hepatoid histomorphology) can have an aberrant expression of albumin.

1683 Necrosis Correlation Between Biopsy and Subsequent Hepatectomy in Fulminant Liver Failure

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Background: Liver biopsy is the gold standard for evaluating hepatic disease, though variation due to sampling and lesional heterogeneity is well documented. In fulminant liver failure with necrosis (FLF), percent necrosis predicts survival, which often guides transplantation decision-making. To access the reliability of liver biopsy in FLF, this study correlated necrosis and collapse (NC) in biopsies with subsequent hepatectomies from orthotopic liver transplantation (OLT) patients.

Design: Institutional records from 2004 through 2015 were searched for all liver biopsies showing >25% NC. Biopsies with a proceeding hepatectomy for OLT available for review were selected. Necrosis due to focal lesions was excluded, as well as cases with a biopsy to resection (BX-RXN) interval >30 days or an etiologic event occurring in the interim. Clinical information was collected including patient history, admission laboratory values, hepatic imaging proximal to biopsy, and procedural information. Percent NC was calculated for each biopsy and resection. Gross and histologic findings were analyzed for clinicopathologic correlations. The end points were absolute BX-RXN percent NC discrepancy (ABRD) and patient death.

Results: Twenty-three cases met the study criteria (1 cirrhotic liver and 5 allografts). Sixteen patients were female. Average age was 37 years. FLF etiologies included 6 ischemia, 5 acetaminophen toxicity, 5 autoimmune hepatitis, 2 hepatitis B virus, 1 hepatitis A virus, and 4 cryptogenic. FLF presentations were 12 hyperacute, 7 acute, and 4 subacute. Eight patients died after OLT (7 during the same hospitalization). Laboratory values and imaging appearances varied widely with no significant correlation with the end points. Biopsy types were 12 transjugular, 5 laparoscopic, 2 percutaneous, and 3 wedge. Average core length was 23 mm (range 3-34 mm, 70% ≥19 mm). Average BX-RXN interval was 5 days (range 1-23 days). Average NC for biopsy and resection was 81% (range 30-100%) and 71% (range 20-95%), respectively. Average ABRD was 19% (range 5-65%). ABRD was directly proportional to BX-RXN interval, but this association was negligible when the interval was ≤7 days. In this study, biopsy length and type did not significantly affect ABRD.

Conclusions: In FLF with necrosis, histological correlation between liver biopsy and subsequent hepatectomy is largely well matched, especially when the biopsy is taken within one week prior to OLT. Regardless of the acquisition approach, an adequate liver biopsy is reliable for evaluating percent necrosis to facilitate decision-making.

1684 Inhibition of Cyclic Adenosine Monophosphate (cAMP)-Response Element-Binding Protein (CREB)-Binding Protein (CBP)/β-Catenin Directly Attenuates the Hepatocytes-Mediated Fibrogenesis

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Background: β-catenin-mediated signaling of Wnt/β-catenin pathway is involved in hepatic fibrosis, and may become a molecular target of the antifibrotic treatment for liver cirrhosis. A chemical compound, PRI-724 developed by PRISM Pharma, selectively inhibits the cAMP-response element-binding protein-binding protein (CBP)/β-catenin interaction in Wnt/β-catenin pathway and restrains the cellular proliferation of both normal and cancer stem/progenitor cells. Although the *in vivo* study using rodent model of hepatic fibrosis have already revealed the effects of PRI-724 on hepatic stellate cells (HSCs) and macrophages (Osawa et al, EBioMedicine, 2015), the direct action of PRI-724 on hepatocytes remains unclear.

Design: To determine this issue, the immortalized human hepatocytes (OUMS29) were treated with PRI-724 (10μM, 24hrs) and then mRNA samples were analyzed by the cDNA array (Agilent Array).

Results: PRI-724 treatment did not affect the expression levels of major Wnt/β-catenin signaling molecules such as *CTNNB1* (β-catenin), *CREB1* (CBP), or *EP300* (p300), but significantly decreased those of some Wnt/β-catenin target genes. The mRNA expression of epithelial-mesenchymal transition (EMT)-related protein S1004A and fibrogenic factor connective tissue growth factor (CTGF) and COL6α1 were decreased by PRI-724 treatment. In addition, the increases of Homo sapiens CCAAT/enhancer binding protein (C/EBP) α transcript variant 1 promoting the maturation of hepatocytes is found in treated hepatocytes. Homo sapiens chemokine (C-C motif) ligand 2 (CCL2) associated with chemoattraction of HSCs was deceased.

Conclusions: In conclusion, PRI-724 could directly affect hepatocytes in addition to HSCs and macrophages and provide the anti-fibrotic effects via the inhibition of hepatic EMT, fibrogenic factors, and HSCs attraction along with the promotion of hepatocellular maturation.

1685 Morphological Features of Early Acute Hepatitis Following Liver Transplantation for Hepatitis C Disease

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Background: End stage liver disease due to hepatitis C virus (HCV) infection is still the leading indication for liver transplantation (LT) in Europe and United States. Fibrosing cholestatic hepatitis (FCH) is a rare and severe form of early recurrence after LT, which rapidly results in graft loss. Its clinical diagnosis can be challenging and in some cases it remains based on histopathological assessment.

Design: Clinical and laboratory data from patients who underwent LT for HCV-related end-stage liver disease in 2 LT centers (Hospital Clínic in Barcelona and Padua University Hospital) from 2000 to 2015 and who developed acute hepatitis in the first 8 months after LT were collected retrospectively. Liver biopsies were evaluated for the following data: ballooning (absent, mild, moderated or marked), lobular disarray (absent, focal or diffuse), ductular reaction (absent or present in less or more than 50% of portal tracts), cholestasis (absent, focal or diffuse), perisinusoidal fibrosis (absent, focal or generalized) and necroinflammation (grade and localization).

Results: A total of 132 patients were included. They were divided according clinical and laboratory data into 3 categories: acute hepatitis (AH), cholestatic acute hepatitis (CAH) and FCH. Biopsies from 73 patients (14 AH, 34 FCH and 25 CAH) were reviewed independently by 2 pathologists. Ballooning, lobular disarray and necroinflammation were present in the majority of cases (89%, 91% and 94%, respectively). However, while ballooning was moderated or marked in 19 out of 34 FCH cases, it was absent or mild in 10 out of 14 AH cases and 15 out of 25 CAH cases (P=0.05). We also found statistically significant differences regarding ductular reaction, which affected the whole circumference of more than 50% of portal tracts in 17 out of 34 FCH cases, 3 out of 14 AH cases and 3 out of 25 CAH cases (P=0.017). No statistically significant differences were found regarding cholestasis or perisinusoidal fibrosis, which are also considered classical hallmarks of FCH. Based on histological criteria, 6 patients (1 from AH group and 5 from CAH) were re-classified as FCH.

Conclusions: Marked hepatocyte injury, severe cholestasis, ductular reaction and perisinusoidal fibrosis are considered histopathologic hallmarks of FCH. However they are non specific and other non-viral causes of liver allograft dysfunction must be included in the differential diagnosis. Clinical history is extremely helpful. Nonetheless we should take into consideration that, in some cases, clinical and laboratory data fail to suspect FCH.

1686 Characterization of Primary Human Hepatic Stellate Cells

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Background: In response to chronic liver injury, secreted cytokines, including TGF-b, activate quiescent hepatic stellate cells (HSCs) into collagen producing myofibroblasts resulting in hepatic fibrosis. HSCs isolated from resected tissue behave abnormally and do not survive as a result of the hypoxic tissue environment. Contrarily, HSCs isolated from disqualified transplant livers are healthy and able to passage, as the tissue is well perfused, preserving the cells in a physiological environment. The goal of this study is to utilize disqualified transplant livers to produce a well-characterized profile of both healthy and diseased phenotypes useful for future studies of liver disease.

Design: H&E and Trichrome slides were reviewed and a pathology report was produced for each case. Donors were classified as either "Healthy" or "Diseased" based on their degree of fibrosis, steatosis, and NAFLD activity score. The expression levels of pro-fibrogenic genes hACTA2, hCOL1a1, hTGFb, hTIMP1, hPDGFRb, and hLOXL2 in the healthy and diseased donor groups were analyzed by RT-qPCR and then compared. Sectioned tissue was stained for a-SMA and Desmin. Isolated HSC cultures were characterized by immunocytochemistry staining for GFAP, Desmin, and a-SMA (typical markers of activated HSCs). The purity of the cultures was assessed by immunocytochemistry staining for CD-11b, CD-31, and TE-7 (markers of Kupffer cells, endothelial cells, and fibroblasts, respectively).
Results: The healthy donors exhibited lower expression of pro-fibrogenic genes compared to the diseased donor group. Immunohistochemistry staining showed weaker a-SMA staining in the healthy donor group. Desmin positive staining was consistent between the healthy and diseased groups. All HSC cultures stained positive for GFAP, Desmin, and a-SMA, and mostly negative (<3% positive) for CD-11b, CD-31, and TE-7.
Conclusions: Disqualified donor liver are a valuable resource for development of primary human liver cultures. Primary hepatic stellate cell cultures were developed in order to study the process of fibrogenesis in liver. The selected RT-qPCR and immunohistochemistry markers are an effective method for characterization of healthy and diseased HSC phenotypes. Having this comprehensive profile of healthy and diseased phenotypes is an invaluable tool for investigators interested in further study of chronic liver disease.

1639 Isolated Vascular "v" Lesions in Liver Allografts: Appropriate Work-Up for This Puzzling Finding

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Background: Isolated vascular or "v" lesions (i.e., arterial intimal inflammation or sclerosis with minimal other histopathologic findings) have recently been adopted into the Banff Grading Criteria for kidney allograft rejection. These lesions are now a component of the morphologic criteria for diagnosing both T cell-mediated (TCMR) and antibody-mediated (AMR) rejection in kidney allografts. However, the significance of isolated "v" lesions in liver allografts from patients who are otherwise asymptomatic is not known.
Design: We identified 9 patients in our pathology database who received liver transplants between 2000 and 2014 and were diagnosed with isolated "v" lesions; these included cases with intimal inflammation, fibrosis, or both. These patients were all clinically asymptomatic and had normal or near normal liver injury tests. We compared these cases to controls that were not only age, sex, and race-matched, but also matched to length of engraftment and native liver disease. The arterial lesions were further evaluated with CD3 and CD68 to detect arteritis, CD31 to analyze the integrity of the peribiliary vascular plexus, and C4d to detect possible underlying AMR (since it is a component of the diagnostic criteria for AMR in both kidney and liver allografts).
Results: The majority (5/9: 55.6%) of the isolated "v" lesions were confirmed to be arteritis and were positive for CD3 and CD68. Focal or diffuse C4d positivity was identified in 62.5% of the "v" lesion cases versus 11.1% of matched controls. The CD31 staining did not detect a significant difference in the vascular integrity between the "v" lesion cases or controls. One patient progressed to graft failure, another developed otherwise typical moderate to severe TCMR in multiple subsequent biopsies, and one patient was eventually retransplanted.
Conclusions: The presence of isolated "v" lesions in liver allografts should prompt pathologists to further evaluate the case with C4d staining, to suggest correlation with donor specific antibody testing, and to recommend close follow-up for possible impending TCMR or AMR. Since all of the patients with isolated "v" lesions in our study were otherwise asymptomatic, liver biopsies should be evaluated prior to weaning of immune suppressive therapy. We also propose that the presence of isolated "v" lesions should be added as one of the morphologic features in the new Banff criteria for diagnosis of antibody-mediated rejection in liver allografts.

1687 Utility of Copper Staining in Determining the Etiology of Cirrhosis

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Background: Determining the etiology of cirrhosis is often challenging, though it guides patient management and influences prognosis. A small fraction of copper is incorporated into ceruloplasmin and released into the blood; the remainder is excreted in bile. Copper staining has been advocated by some as a marker of an underlying chronic cholestatic condition, while others suggest it accumulates non-specifically with advancing fibrosis. To evaluate the utility of copper staining in our clinical practice, we sought to correlate rhodanine histochemical staining pattern in a large cohort of cirrhotic patients of clinicopathologically defined etiologies.
Design: Copper (rhodanine) staining was performed on 152 cirrhosis specimens of the following etiology: hepatitis C (HCV, n=59), alcoholic steatohepatitis (ASH, 28), non-alcoholic steatohepatitis (NASH, 14), alpha-1 antitrypsin deficiency (A1AT, 13), primary biliary cirrhosis (PBC, 8), primary sclerosing cholangitis (PSC, 7), PSC/autoimmune hepatitis (AIH) overlap (2), AIH (8), and other (13). We also stained a fibrosis progression cohort (83 non-cirrhotic HCV, stages 0-3). Copper staining was graded as follows: 0, none; 1 focal periportal/periseptal; 2, multifocal, multilobular; 3, linear periportal (≥3 adjacent hepatocytes); and 4, lobular (≥3 hepatocytes from limiting plate).
Results: Among all cirrhosis cases, 51% showed at least grade 1 staining. All PBC/PSC cases (n=17), including two with overlap syndrome, showed at least grade 2 staining and usually showed grade 3 or 4 staining (82%). Grade 3 or 4 staining was only seen in 13% of cases of non-cholestatic etiology. Only 2/83 (2%) cases in the fibrosis progression cohort showed any staining (both grade 1). Detailed data for cirrhosis cases are presented in the table.

Diagnosis (n)	Copper Grade (%)				
	0	1	2	3	4
HCV (59)	48	25	10	2	15
ASH (28)	64	21	11	4	0
NASH (14)	72	7	7	14	0
A1AT (13)	31	15	23	23	8
PBC (8)	0	0	25	25	50
PSC (7)	0	0	14	29	57
PSC/AIH (2)	0	0	0	0	100
AIH (8)	88	12	0	0	0
Other (13)	54	38	0	8	0
Total (152)	49	20	10	8	13

Conclusions: Although lower-grade copper staining is etiologically non-specific in cirrhosis, higher-grade staining (grade 3-4) is reasonably sensitive and specific (82% and 87%, respectively) for a chronic cholestatic etiology (e.g., PBC, PSC).

1688 Distinction of Intrahepatic Metastasis from Multicentric Carcinogenesis in Hepatocellular Carcinoma Using Molecular Markers

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Background: Patients with hepatocellular carcinoma (HCC) frequently have multiple anatomically separate tumors. In these patients, multiple hepatocellular carcinomas could represent intrahepatic metastases of a single cancer or multicentric carcinogenesis. The frequency and clinical implications of these two possibilities are currently unknown.
Design: We searched our pathology archives from 2010 to 2015 to identify liver resections and explants with multiple anatomically distinct hepatocellular carcinomas. Clinical information and histological slides were reviewed. Each tumor was evaluated for architectural growth pattern and subclassified into pseudoglandular, solid, trabecular, or clear cell type. In addition, we performed Sanger sequencing to characterize mutations in the TERT promoter region, the most commonly mutated gene region in HCC, in 111 tumors from 37 patients.
Results: C228T and C250T mutations in the TERT promotor region were present in 73% (81/111) and 1.8% (2/111) of tumors, respectively. Histologically 35.1% (13/ 37) of the patients had at least one tumor with a different growth pattern. Comparison of TERT promoter mutations in each patient's tumors showed that 29.7% (11/37) of the patients had discordant TERT promoter mutations in at least one of the tumors, suggesting that at least ~30% of patients with multiple HCCs have multicentric carcinogenesis. Discordant TERT promoter mutations were present in 13.8% of the cases that had similar histology, suggesting that histology alone cannot be used as a surrogate for tumor relatedness. Liver transplant was performed in 65% (24/37) of the patients with a recurrence rate of 25% (6/24). Furthermore, 83% (5/6) of the transplanted cases with recurrence had tumors with concordant TERT promoter mutations, implying that intrahepatic metastasis may have a worse prognosis than multicentric carcinogenesis.
Conclusions: TERT promotor mutation status can be used to differentiate intrahepatic metastasis from multicentric carcinogenesis in hepatocellular carcinoma. However, this strategy likely overestimates relatedness, as the same hotspot mutation could occur independently. Thus, future directions include next generation sequencing of other common mutations in HCC to further evaluate relatedness of multiple tumors.

1689 Post-Transplant Plasma Cell Hepatitis in Primary Sclerosing Cholangitis: A Clinico-Pathologic Study

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Background: Plasma cell hepatitis (PCH) is a rare form of liver graft dysfunction which occurs in liver transplant (LT) recipients of different etiologies other than autoimmune hepatitis. It is characterized by plasma cells comprising >30% of the infiltrate on a liver biopsy, has worse clinical outcome, and typically progresses to end-stage liver disease requiring re-LT. Previous studies on PCH were on post-LT HCV patients. To date, data of PCH occurring in primary sclerosing cholangitis (PSC) patients is unknown. We evaluated the characteristics of PSC patients who developed PCH at our institution.
Design: Pathology database search was performed to identify PCH cases diagnosed at our institution in the last 5 years (2011-2016). Electronic medical records were reviewed to document the original liver disease. Retrospective review of the clinical and pathologic information of PSC patients who developed PCH was performed.
Results: Seventy-four liver biopsies with PCH from 56 patients were identified. Ten of 74 cases (13.5%) belonged to 5 PSC patients (8.9%) who underwent LT. Other original liver diseases included chronic hepatitis C (45.9%), primary biliary cholangitis (8.1%), biliary atresia (6.8%), fulminant hepatic failure (6.8%), Wilson's disease (4%), and others (14.8%). A total of 58 patient-years from 5 PSC patients were evaluated in this analysis. Most episodes of PCH coincided with elevated ALT (84.6%, mean = 151.8 U/L), AST (85.7%, mean = 157.3 U/L), alkaline phosphatase (71.4%, mean = 253 U/L), and GGT levels (81.8%, mean = 158.27 U/L).

Patient	Sex	Age of LT (years)	Interval LT to PCH (months)	ACR prior to PCH	Recurrent PSC	Re-LT	Status
1	F	26	48	Yes	Yes	No	Living
2	F	36	3	Yes	No	Yes	Living
3	M	53	29	No	No	No	Living
4	F	59	120	No	Yes	No	Living
5	F	58	140	No	Yes	No	Deceased

Conclusions: In the post-LT setting, PCH is an important diagnostic consideration in PSC patients who present with elevated liver enzymes. PCH may present either early or late in these patients. Moreover, PSC recurrence is common and only a small number of patients require re-LT.

1690 Histologic Assessment of HCV Liver Transplant Recipients with Sustained Virologic Response After Treatment with Direct-Acting Antiviral Agents

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Background: Recent development of direct-acting antiviral agents (DAAs) has transformed the management of patients with HCV and treatment strategies are associated with high sustained virologic response (SVR) rates. The aim of the study was to correlate the histologic changes with SVR status of HCV patients (SVR+) who received DAAs compared to those who did not receive DAAs (control) prior to liver transplantation (LT).

Design: Fifty-eight adult HCV patients underwent LT at our institution from 2014-2016 were included with 25 SVR+ comprising the study group and 33 for the control group. Two pathologists, blinded to clinical information, evaluated the histologic sections simultaneously with 4 H&E stained representative slides evaluated per case. Assessment included the histology activity index (HAI/Knodell score), fibrosis (Ishak score), and stage of Laennec cirrhosis. SVR status of each patient was assessed by a transplant hepatologist. Correlation was determined using Fisher's exact test.

Results: There was no significant difference in age and gender between the 25 SVR+ patients (M:F=1.3:1, ave age:63.8 years) and 33 control patients (M:F=2.3:1, ave age:61.7 years). There was no significant difference in the HAI of both groups (p=0.62), as most patients in both groups showed minimal (HAI:1-4) to mild inflammation (HAI:5-8). Patients who achieved SVR also did not show less portal inflammation (p=0.61), interface hepatitis (p=0.87), confluent necrosis (p=0.71), or spotty lytic necrosis (p=0.69) compared to the control group. All patients had cirrhosis and the majority of patients who received LT had Laennec 4B (22.4% 4A, 65.6% 4B, and 12% 4C). Interestingly, 11 patients were identified to have marked cholestatic changes. Nine of the patients (81.8%) belonged to the group of patients who did not achieve SVR.

Conclusions: Histologic necroinflammatory activities and fibrosis characteristics of HCV patients who received DAAs do not correlate with their SVR status. This confirms the observation that chronic inflammation persists despite the absence of the virus, supporting the notion that inflammation is immunologically driven. Moreover, cholestatic changes are more prevalent in patients who did not achieve SVR, which might be used as a marker of treatment failure in some HCV patients. Finally, liver failure necessitating LT develops in HCV patients with all stages of Laennec cirrhosis, and not just in those with markedly shrunken livers.

1691 Hepatocellular Carcinoma in Primary Sclerosing Cholangitis: 10-Year Experience at a Transplant Center

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Background: Primary sclerosing cholangitis (PSC) is associated with an increased risk of cholangiocarcinoma (CCA) with an incidence up to 15%, while the risk of hepatocellular carcinoma (HCC) in these patients is reported to be extremely low. Zenouzi et al. examined explants from 140 patients who underwent liver transplantation (LT) for PSC and found no cases of HCC, concluding that HCC surveillance was not warranted in these patients (Clin Gastro Hepatol 2014). We have identified cases of HCC in PSC patients at our institution. We sought to determine the incidence of HCC in PSC patients undergoing LT at our institution and characterize these lesions.

Design: Pathology database review to identify PSC patients who underwent LT from 2006 to 2016 was performed. Electronic medical records were reviewed to analyze the clinical and pathologic characteristics of HCC cases which developed in these patients. Patients younger than 18 years of age and those with concomitant liver diseases were excluded from the study.

Results: Forty-six PSC patients (M:F=1.3:1, mean age of 49.8 years) underwent LT at our institution during a 10-year period. Thirty-nine cases (84.8%) showed cirrhotic liver and the remaining 7 cases (15.2%) showed bridging fibrosis. HCCs were identified in 6 patients (13%), all with cirrhosis. These patients were older at the time of LT (mean age of 66.8 years) and predominantly male (M:F=2:1). Two of these patients had inflammatory bowel disease (1 Crohn's disease and 1 ulcerative colitis). HCCs were identified in 5 patients prior to LT, while one patient with 2 small HCCs and a perihilar CCA was not detected prior to LT. Thirteen HCC lesions from 6 patients were analyzed in this study (5 patients with multiple lesions). The size of the lesions ranged from 0.7 to 4.2 cm (mean=2.0 cm). The lesions were well (46.1%) or moderately differentiated (53.9%). Microscopic vascular invasion was identified in 2 lesions (15.4%). All 6 patients presented with either pT2 N0 (33.3%) or pT2 Nx (66.7%) tumor stage at the time of LT (AJCC 7th edition).

Conclusions: We found that HCC occurred in 13% of PSC patients who underwent LT at our institution HCCs in these patients are often multiple but they usually show favorable characteristics (small, well-to-moderate differentiation, and without vascular invasion). The high incidence of HCC in in PSC patients undergoing LT in our study indicates importance of HCC surveillance in these patients.

1692 A Point-Based Grading System for Hepatocellular Carcinoma Shows Improved Interobserver Agreement and Better Identifies Histologic Features Associated with Recurrent Disease After Liver Transplant

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Background: The most widely-accepted criteria to determine liver transplant candidacy in the setting of hepatocellular carcinoma (HCC) are based exclusively on imaging features. Tumor differentiation has been proposed as a prognostic marker that can refine clinical decision making based on radiographic data (i.e. tumor size and number). However, histologic grading of HCC has historically shown very poor interobserver concordance. This study sought to establish a reproducible grading criteria for HCC, specifically aimed at evaluating pre-transplant liver biopsies in order to improve pre-operative risk stratification.

Design: 112 liver explants performed at our institution between 1997 and 2015 for HCC were examined for histologic features and CK19 immunohistochemical staining. Architectural patterns, cytologic features, and CK19 expression were scored in each case, and then analyzed for correlation with tumor recurrence following transplantation. Using this, we developed a point-based grading system: 1 point for scirrhous growth, 1-2 points for nuclear atypia, 1 point for amphophilic cytoplasmic, 1 point for CK19 positivity.

Results: We found that tumor recurrence correlated with architecture, nuclear-to-cytoplasmic ratio, degree of nuclear atypia, cytoplasmic quality, and CK19 expression ($p < 0.05$). A point-based grading system using these variables significantly improved outcome stratification compared to the traditional Edmonson grading system and also significantly improved interobserver agreement on tumor differentiation.

Comparison of Grading Criteria			
	Recurrences	Total Cases	Percentage
Edmonson System			
Well	0	45	0.0%
Moderate	12	58	20.7%
Poor	3	9	33.3%
Point-Based System			
Well	0	40	0.0%
Moderate	8	61	13.1%
Poor	7	11	63.6%

Conclusions: Our previous research has shown that pre-transplant liver biopsy significantly improves risk stratification among transplant patients compared to imaging alone. In a retrospective analysis, we identified histologic characteristics of HCC that cluster among recurrent tumors and used these variables to construct a point-based grading system, which shows improved interobserver agreement compared to traditional Edmonson grading. Application of our point-based grading system may allow for more accurate risk stratification and a more individualized approach to transplant eligibility and priority.

1693 Feasibility and Utility of In-Situ Hybridization for Micro-RNA 21 in Intrahepatic Cholangiocarcinoma

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Background: Histological distinction between benign and malignant biliary proliferations in liver can be challenging, particularly with small biopsies. There is no immunohistochemical (IHC) marker which can distinguish them reliably. Micro-RNA (miRs) are not translated, and therefore cannot be detected by IHC, but are known to show differential expression patterns in benign and malignant tissue. MiR-21 is strongly associated with malignancies, and molecular studies have shown it to be present in cholangiocarcinoma (ChCa). Its detection has never been attempted in formalin fixed paraffin embedded (FFPE) liver tissue.

Design: Detection of miR-21 by in-situ hybridization (ISH) was performed using the View RNA ez-L Kit (Affymetrix, Santa Clara, CA) on the Leica Bond III on tissue microarrays (TMAs) including 39 cases of ChCa and 65 cases of benign bile ducts (43 cases of biliary obstruction and benign ducts from 22 ChCa cases). Scoring included both percentage of overall staining within the biliary epithelium and manual semi-quantification of miR-21 signals per cell. Percentage was scored as follows: 1 = 1-33% staining, 2 = 34-66% staining, 3 = 67-100%. Mir-21 signals per cell were scored as follows: 0 = <3 signals per cell, 1 = 3-5 signals per cell, 2 = 5-10 signals per cell, 3 = >10 signals per cell. The final score for each case was calculated by summing both scores. Total scores of 3 or greater were considered positive.

Results: The average miR-21 score was 4.54 in ChCa and 0.47 for benign cases (p<.0001). 36 of 39 ChCa cases were considered positive compared to 2 of 65 benign controls. The sensitivity and specificity of mir-21 ISH for detection of ChCa was 92% and 97%.

Conclusions: Micro-RNAs can be detected in FFPE liver tissues by ISH on an automated platform. These may be useful in diagnostic, prognostic, and investigational settings. Here we report that MiR-21 distinguishes benign from malignant biliary cells with high sensitivity and specificity.

1694 Activity of Autoimmune Hepatitis in Explanted Livers Correlates with Acute Cellular Rejection and Disease Recurrence During First Year Post Transplantation

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Background: Liver transplantation is an accepted therapeutic modality for patients with fulminant liver failure and end-stage liver disease due to autoimmune hepatitis (AIH). Patients with AIH are at an increased risk for acute cellular rejection and disease recurrence in the allografted liver. In this study, we correlated activity of autoimmune hepatitis in explanted livers with disease recurrence and acute cellular rejection within the first year post transplantation.

Design: Sections from explanted livers were reviewed by two pathologists (NR, RS). Disease activity was categorised as shown in table 1. Information about HLA donor-recipient mismatch was available only in a few cases precluding any corrrelations.

Table 1: Histologic Categorization of Disease Activity in Liver Explants

Histologic Category	Interface activity	Collapse	% Area of collapse
I	None to mild	No	None
II	Moderate to severe	No	None
III	Moderate to severe	Yes	<50
IV	Moderate to severe	Yes	≥50

Results: There were 31 patients (10 males: 21 females, age range 22-69 year old) who underwent liver transplantation for decompensated liver disease due to AIH. The MELD scores at the time of transplantation were available for 21 patients (68%). A third of patients required biopsy of the allograft for management of abnormal liver tests during the first year of transplantation. Table 2 correlates the histopathologic categories with average MELD score at time of transplanation, incidence of acute cellular rejection and disease recurrence during the first year post transplantation.

Table 2: Correlation of Disease Activity in Explants with MELD score, Disease Recurrence and Acute Cellular Rejection

Histologic Category	Number of patients (%)	MELD score at transplantation	Recurrence within first year post transplantation (%)	Acute cellular rejection within first year post transplantation (%)
I	18 (58)	22	3(17)	1(6)
II	4 (13)	25	None	2(50)
III	7 (28)	22	None	3(43)
IV	2 (6)	33	1(50)	1(50)

Conclusions: Higher MELD scores were seen in patients with category IV disease activity which correlates pathologically with submassive necrosis and clinically with fulminant hepatic failure. These patients are also at risk for disease recurrence and acute cellular rejection in the allograft within the first year of transplantation.

The incidence of acute cellular rejection is low with patients with no or mild disease activity (category 1).

1695 SOX-9 Is Superior to Other Cancer Stem Cell Markers EpCAM and CK19 in Predicting Prognosis in Western Hepatocellular Carcinoma

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Background: Various cancer stem cell markers, such as epithelial adhesion molecule (EpCAM) and cytokeratin19 (CK19), have been proposed to be associated with poor prognosis in hepatocellular carcinoma (HCC), particularly in Asian HCC cohorts. We have previously shown that sex-determining region Y–box 9 (SOX-9) protein is a marker of biliary differentiation and frequently expressed in primary liver carcinoma with biphenotypic differentiation. Studies in Asian HCC cohorts also suggest that SOX-9 is a liver progenitor cell marker and is associated with worse outcome in HCC patients in Asia. Since predominant epidemiology and pathogenesis of HCC are different in Western and Asian populations, we aimed to determine the expression and prognostic significance of SOX-9 and other cancer stem cell markers in Western HCC population.

Design: Tissue microarray was constructed using 224 resected or transplanted pure, non-fibrolamellar HCC samples identified in the databases of the Departments of Surgery and Pathology and Immunology at Washington University School of Medicine. Medical records were reviewed, and pertinent demographic and clinicopathological data were extracted. Immunohistochemistry studies for EpCAM (Millipore, 1:1000), CK19 (Cell Marque, 1:100) and SOX-9 (Cell Marque, 1:800) were performed and scored in a semiquantitative manner. Immunohistochemistry results were correlated with recurrence-free and overall survival using Kaplan-Meier method and statistical analysis was performed using the Log rank test and Z score test.

Results: The prevalence of SOX-9 expression (14% of cases) was similar to that of CK19 (15%, p = 0.73), but significantly less frequent than that of EpCAM (33%, $P<0.01$). Nuclear SOX-9 expression in HCC correlated significantly with both reduced overall survival ($P = 0.048$) and recurrence-free survival ($P< 0.001$). In contrast, neither EpCAM nor CK19 expression showed significant correlation with overall or recurrence-free survival (P values: 0.078-0.63).

Conclusions: Among the three HCC cancer stem cell markers tested, only expression of SOX-9 correlated with poor outcome in Western HCC cohort. Therefore, SOX-9 expression is a reliable prognostic biomarker for HCC survival across ethnic groups and different etiologies.

1696 CCL2/CCR2 Axis Is Involved in Hepatocellular Carcinoma

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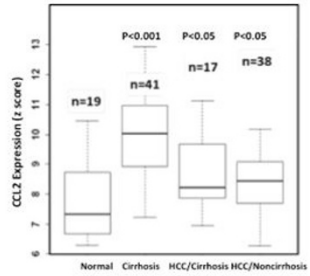
Background: C-C motif chemokine ligand 2 (CCL2/MCP1) is a chemokine that is responsible for recruiting chemokine receptor 2 (CCR2)-positive immune cells, which subsequently produce pro inflammatory cytokines, including interleukin-6 and tumor necrosis factor-α. CCL2 is involved in the pathogenesis of several inflammatory diseases, and promotes inflammation and tumorigenesis in the liver. Additionally, the availability of CCR2 antagonists makes this axis an important possible therapeutic target for hepatocellular carcinoma (HCC). Murine studies have shown CCR2 antagonists decrease HCC. Our goal is to determine if CCL2 RNA is increased in human HCC, and if protein expression correlates with patient demographics and/or tumor characteristics.

Design: In order to evaluate CCL2 expression in human liver, we analyzed HCC RNA microarray data (n=115, mainly Hepatitis C virus positive) from GEO (GSE14323) using ANOVA. Subsequently, we identified 198 cases of HCC from our archives from 1997 to 2016. Patient age, sex, tumor grade and size were noted. Tumor microarray was constructed using 2, 1 mm cores per case. Sections were immunostained with CCL2 and CCR2, and intensity was graded as 0 (absent), 1 (weak), 2 (moderate), or 3 (strong).

Results: The analysis of gene expression profiling data downloaded from the public database, GEO, showed that CCL2 RNA level was significantly elevated in HCCs and cirrhotic livers compared to normal livers.



CCL2 staining was present in 184 (93%) and absent in 14 (7%) cases of HCC. The intensity was graded 1 in 50 cases, 2 in 112 cases and 3 in 22 cases. Intensity did not correlate with tumor grade, size, or patient age, but it positively correlated with male gender. Inflammatory cells in almost all cases stained strongly for CCR2.

Conclusions: CCL2 RNA is increased in cirrhosis and HCC suggesting that CCL2 might be important for progression to cirrhosis and HCC. CCL2 and CCR2 protein expression were common in our HCCs, but intensity did not correlate with tumor characteristics we evaluated. Interestingly, CCL2 protein expression correlated with male gender and HCC is most common in men. Since there are antagonists available for the CCL2/CCR2 axis, these findings warrant additional studies to evaluate this pathway and antagonists.

1697 Arginase-1 (Arg-1) Is a Specific but Not Sensitive Immunohistochemical Marker for Hepatocellular Carcinoma (HCC)

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Background: The distinction of hepatocellular carcinoma (HCC) from metastatic tumor in the liver, and metastatic HCC to other organs often presents a diagnostic challenge. The number of diagnostically useful immunohistochemical markers for hepatocytes is limited to hepatocyte paraffin antigen (HepPar-1), polyclonal carcinoembryonic antigen, and CD10, with α-fetoprotein and glypican-3 labeling HCCs. Arginase-1 (Arg-1) is a binuclear manganese metalloenzyme involved in the urea cycle that catalyzes the hydrolysis of arginine to ornithine and urea. Therefore, we investigated the expression of Arg-1 in benign and malignant hepatocytes as well as other common tumors.

Design: We constructed tissue microarrays (TMA) from 120 cases of HCC with paired adjacent non-neoplastic tissue (36 well-differentiated, 53 moderately-differentiated, and 31 poorly-differentiated HCC), 110 cases of different types of renal cell carcinoma (RCC) with paired adjacent non-neoplastic tissue, 91 cases of different types of ovarian tumor, 44 cases of urothelial carcinoma, 27 cases of prostatic adenocarcinoma, 22 cases of lung adenocarcinoma, and 14 cases of colon adenocarcinoma. TMA slides were subjected to Arg-1 immunostaining. For each case, Arg-1 staining was carefully examined along with pathological features such as histological type and grade.

Results: There was a decrease in Arg-1 staining in all histological grades of HCC as compared to adjacent non-neoplastic liver. Arg-1 expression was only observed in ~57.5% of all HCC cases (~56% in well-differentiated, ~62% in moderately-differentiated, and ~52% in poorly-differentiated HCC) as compared to >90% in adjacent non-neoplastic liver.

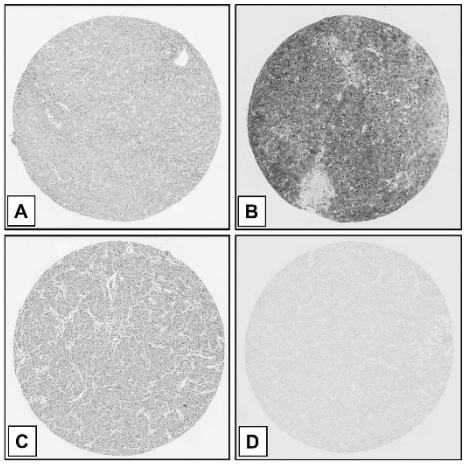

Figure. Representative Tissue microarrays.
(A) H&E non-neoplastic liver. (B) ARG-1 staining non-neoplastic liver. (C) H&E HCC. (D) ARG-1 staining HCC.

All cases of RCC with paired adjacent non-neoplastic tissue, ovarian tumor, urothelial carcinoma, prostatic adenocarcinoma, lung adenocarcinoma, and colon adenocarcinoma were negative for Arg-1 staining.

Conclusions: Compared to normal hepatocytes, most HCC cells had either loss or decrease of Arg-1 expression. There was no significant association between decrease in Arg-1 staining and HCC tumor grade. Our findings show that Arg-1 is not a sensitive marker for HCC, whereas it is a specific marker for HCC and can be a useful diagnostic marker in surgical pathology practice.

1698 Adenovirus Hepatitis: Analysis of 12 Consecutive Cases at a Single Institution

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Background: Although adenovirus is a common, usually self-limited pathogen, in the immunocompromised host it can cause severe infections involving multiple organs including the liver.

Design: The pathology database at a major academic medical center was searched for confirmed cases of adenovirus hepatitis from 1995 to 2016 including biopsy and autopsy specimens. The medical record was reviewed for clinical information. Cases were included only if there was histologic evidence of liver damage and immunohistochemical or laboratory evidence of active adenovirus infection.

Results: Twelve cases of adenovirus hepatitis were identified. There were 8 pediatric patients, 7 of which had received orthotropic liver transplants and 1 of which was receiving chemotherapy for acute lymphoblastic leukemia. There were 4 adult patients, of which 1 was actively receiving chemotherapy for chronic lymphocytic leukemia and 2 had undergone hematopoietic stem cell transplantation for hematologic malignancies. One patient had lymphoplasmacytic lymphoma and had received chemotherapy over a year prior but was not currently receiving therapy. In all cases, histologic sections showed coagulative hepatocyte necrosis and characteristic nuclear inclusions. Hepatocyte necrosis ranged from spotty to massive. Although this necrosis was predominantly non-zonal, half of the cases demonstrated a tendency for periportal necrosis. The majority of cases (7/12; 58%) cases had no associated inflammation. If present, inflammation was focal and lymphohistiocytic. Interestingly, histologic changes showed some anatomic heterogeneity within the liver. In one case, a biopsy of the peripheral liver showed only focal portal lymphocytic infiltrates and rare granulomas while a simultaneous image-guided biopsy of a liver lesion showed massive necrosis with viral inclusions. Among the pediatric patients, 63% (5/8) died secondary to organ failure, while there was 100% (4/4) mortality in the adult population.

Conclusions: Adenovirus hepatitis is a rapidly progressive and highly lethal infection in the immunocompromised host. In the pediatric setting, infections were most common in patients who had undergone liver transplantation. In contrast, all of the adults had hematologic malignancies and had received either chemotherapy or undergone hematopoietic stem cell transplantation. Histologic findings are characterized by coagulative necrosis and characteristic nuclear inclusions. The majority of cases have minimal or no associated inflammation. Findings can be focal within the liver, sometimes requiring targeted biopsies.

1699 Current Diagnostic Thresholds for Acute Rejection in Liver Allograft Biopsies Are Adequate

Kurt Schaberg, Neeraja Kambham, Richard Sibley, John P Higgins. Stanford University School of Medicine, Stanford, CA.

Background: Liver allograft biopsies may show mild nonspecific changes (e.g., focal, mild portal lymphocytic infiltrates) of uncertain significance that do not meet the threshold for definitive diagnosis. At our institution, these biopsies often receive a diagnosis of "mild nonspecific findings." For this study, we obtained follow-up data on these patients to determine outcome

Design: We identified all liver allograft biopsies at our institution from a 12 year period (2003-2014) that had been signed-out as showing "mild nonspecific findings" or an equivalent diagnosis and that had at least one subsequent repeat biopsy. The index and follow-up biopsies were reviewed by two pathologists. Clinical background information was obtained from the medical record. We also identified a control group of 62 liver allograft biopsies with either no significant abnormality or an alternative diagnosis of low severity (e.g., mild preservation injury) that also had at least one subsequent repeat biopsy. Acute rejection was identified using the Banff schema for grading liver allograft rejection.

Results: 83 liver allograft biopsies with mild nonspecific findings were reviewed in total (40 adult, 43 pediatric). Of the 43 pediatric biopsies, repeat biopsy in less than 1 year showed acute rejection in 11 cases (26%) whereas 3 of 27 pediatric control repeat biopsies showed rejection (11%) ($p=0.14$). Of the 40 adult biopsies, repeat biopsy showed recurrent hepatitis C in 18 cases (45%) compared to 7 of 32 adult control biopsies (22%) ($p=0.049$) and acute rejection in only 5 cases (7%) compared to 8 of 32 adult control biopsies (25%) ($p=0.22$).

Conclusions: In adults, mild nonspecific findings including focal, mild portal lymphocytic infiltrates that do not meet the diagnostic threshold for acute rejection are not associated with acute rejection on repeat biopsy. Instead, these findings are significantly associated with recurrent hepatitis C. These nonspecific findings may represent an early manifestation viral infection in these cases. Although an increased number of pediatric transplanted livers showed acute rejection on repeat biopsy, this did reach statistical significance. As such, current diagnostic thresholds for acute rejection in both the pediatric and adult setting are adequate as these sub-threshold changes do not appear to progress to acute rejection on repeat biopsy.

1700 Histologic Progression of Ischemic Cholangiopathy in Liver Allografts of Donation After Cardiac Death

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Background: In the last decade, donation after cardiac death (DCD) liver grafts have been identified as one approach to alleviating the donor organ shortage. Utilization of DCD grafts, however, has been hindered by lower graft survival rates and higher biliary complication rates in comparison with donation after brain death (DBD) liver grafts, mostly attributed to ischemic cholangiopathy (IC). The histologic progression of IC in DCD allografts has not been fully characterized and early changes may have been under-recognized. We aim to characterize the histologic evolution of IC in DCD allografts.

Design: The study includes (1) DCD liver allografts that developed IC (n=10), (2) DCD liver allografts without IC (n=10) and (3) DBD liver allografts without IC (n=10). IC was confirmed by imaging. In group 1, allograft biopsies at time zero, interim (mean 48 days post transplant) and time of diagnosis of IC by imaging (mean 484 days post transplant), and time-matched allograft biopsies in group 2 and 3 were reviewed blindly by a study pathologist.

Results: Biopsies at the time of IC confirmation by imaging were most striking for moderate to severe sinusoidal dilatation, severe bile ductular reaction, severe damage of bile ducts, severe portal edema, and portal/periportal fibrosis. None of the biopsies in groups 2 and 3 at the same time point showed these changes. Interim biopsies of the IC group showed mild to moderate centrizonal hepatocellular dropout, moderate sinusoidal dilatation, and variable degree of bile ductular reaction and bile duct damage. Again, none of the biopsies in groups 2 and 3 at the time point showed these changes. Time zero biopsies of the IC group showed moderate to severe preservation injury, while those of group 2 only showed minimal to moderate preservation injury. Preservation injury was minimal in group 3.

Conclusions: Sinusoidal dilation, bile ductular reaction, damage of bile ducts, portal edema, and portal/periportal fibrosis are the histologic changes of IC in DCD liver allografts. Centrizonal hepatocellular dropout, sinusoidal dilatation, ductular reaction and bile duct damage were also seen as early as 1-2 months after transplant, well before IC is confirmed by imaging. Recognition of these features may aid in the early diagnosis of IC, and severe preservation injury in time zero biopsies may predict the development of IC in DCD allografts.

1701 Serum Amyloid A (SAA) and C-Reactive Protein (CRP) Are Commonly Positive in Hepatocellular Carcinoma and Expression Levels Stratify According to Etiology and the Absence of Cirrhosis

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Background: Inflammation-related signaling pathways are strongly linked to carcinogenesis. SAA and CRP have been described as prognostic serologic biomarkers for hepatocellular carcinoma (HCC). Recently, immunohistochemistry (IHC) for these markers has been reported in some cases of HCC, but the associations have not been fully characterized.

Design: SAA and CRP IHC was performed on FFPE cirrhotic HCC (HCC-C, n=32), non-cirrhotic HCC (HCC-NC, n=29), and non-neoplastic cirrhosis controls (n=21) from patients with NASH/ASH and viral hepatitis (VHep). All tumor samples contained adjacent non-tumor ("background"). Staining intensity was graded from 0-3+, and each grade was multiplied by the corresponding percentage, yielding a numeric expression level score ranging from 0 – 300. A commonly used cutoff value of moderate staining (grade 2) involving ≥ 10% of the tissue (expression level score of ≥ 20) was considered positive.

Results: 62% of HCCs were positive for CRP, but only 38% of HCCs were positive for SAA. The adjacent background tissue was positive for both CRP and SAA in 62% of cases. The non-neoplastic cirrhotic controls were positive for CRP in 95% and SAA in 86% of cases.

Tumor SAA showed higher expression levels in NASH/ASH versus VHep ($p < 0.0001$); tumor CRP did not correlate with etiology. Tumor SAA expression was higher in HCC-

NC versus HCC-C ($p = 0.014$); tumor CRP did not correlate. Tumor SAA tended to be higher in males, but this was not statistically significant. Background tissue adjacent to tumor showed higher SAA levels in NASH/ASH versus VHep ($p < 0.0001$); CRP did not correlate. Background tissue adjacent to tumor showed higher SAA levels in the HCC-NC cases versus the HCC-C cases ($p < 0.0001$); CRP did not correlate. Neither SAA nor CRP in background tissue adjacent to tumor correlated with gender.

Conclusions: SAA and CRP IHC are frequently positive in HCC, adjacent background liver, and non-tumor- bearing cirrhosis. Although both markers are commonly considered similar components of inflammation-mediated signaling that has been coupled to hepatocarcinogenesis, IHC expression levels in both HCC and adjacent background liver stratify differently according to underlying liver disease etiology and the absence or presence of cirrhosis. These findings may reflect important pathobiologic differences.

1702 Frozen Section Artifact May Hinder Assessment of Steatosis in Pretransplant Liver Biopsies

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Background: Frozen section analysis has been routinely used in analysis of livers for organ donation, including determination of the degree of steatosis. While 'frozen section artifact' is often discussed anecdotally, it is not well described in the literature. This artifact may lead to diagnostic challenges, especially for pathologists who do not frequently encounter liver pathology.

Design: We identified 21 cases of pretransplant liver biopsy specimens in our Surgical Pathology archives (May 2012-Oct 2015) where intraoperative diagnosis was rendered by a pathologist not specializing in GI/Liver pathology, with final diagnosis rendered by a GI/Liver pathologist. Concordance between frozen section and final diagnosis was examined. Next, frozen section artifact was prospectively analyzed in four non-cirrhotic liver resection specimens removed for either colorectal (n=2) or hepatocellular carcinoma (n=2). Core biopsy specimens were taken from fresh non-neoplastic background liver parenchyma. Frozen section analysis was performed on these core biopsies at time intervals from 0-24 hours; tissue cores not immediately frozen were placed in saline solution. Patient age, gender, pathologic diagnosis, degree of steatosis, and artifactual morphologic changes were noted.

Results: Five (23.8%) of 21 pretransplant liver biopsy specimens had at least a 10% difference in the reported amount of micro and/or macrovesicular steatosis between intraoperative diagnosis and final report, with the greatest differences noted in the assessment of microvesicular steatosis. The four resection specimens demonstrated 0-10% macrovesicular steatosis with no appreciable microvesicular steatosis on non-frozen H&E-stained sections. Tissue cores which underwent immediate frozen section (time=0) had mild vacuolization of the cytoplasm, while those frozen after 1-4 hours in saline demonstrated increased cytoplasmic vacuolization. Tissue samples that were in saline for 24 hours demonstrated diminished cytoplasmic vacuolization with expansion of the sinusoids. Other artifacts included blade chatter, large holes and variable staining. All frozen section slides had 5-10% macrovesicular steatosis with <5% microvesicular steatosis.

Conclusions: Frozen section artifact is a critical factor in the examination of liver biopsy specimens for organ donation and may vary depending on time in transport media. These artifacts, including cytoplasmic vacuolization, may hinder the appropriate assessment of the degree of steatosis, especially for those who do not routinely examine liver biopsy specimens.

1703 Correlation Between C4d Immunoreactivity and Donor-Specific Antibody in Posttransplant Liver Biopsies Showing Central Perivenulitis

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Background: Central perivenulitis (CP), defined by necroinflammatory changes involving the central veins and zone 3 hepatocytes, is a common histologic finding in posttransplant liver biopsies. If an ischemic etiology and drug injury have been excluded, it is generally thought to be immune-mediated as a manifestation of severe allograft rejection. The role of antibody-mediated mechanisms in the development of CP has not been investigated. In this study, we investigated the relationship of CP with C4d immunoreactivity (C4d-IR) and donor-specific antibody (DSA) status to determine if antibody-mediated mechanisms play a role in the development of CP.

Design: A total of 51 posttransplant liver biopsies (from 51 patients) that showed histologic features of CP were retrieved from our pathology database during a period of 4 years. DSA data were available for 37 cases, which constituted the study group. Detailed clinical history and image findings were reviewed to rule out the possibility of ischemia or drug injury as potential etiologies for CP. Immunohistochemical stains for C4d were performed on formalin-fixed and paraffin-embedded tissue sections and interpreted according to recommendations by the Banff Working Group (*Am J Transplant*. 2016; Epub ahead of print).

Results: Among the 37 cases included in the study, 11 (30%) showed positive C4d-IR in endothelial cells lining the portal veins. Positive staining was also observed in portal stroma, portal capillaries, and focally in sinusoids in a few of these cases. No positive staining was detected in central veins or zone 3. The remaining 26 cases were considered C4d negative. DSA was positive in 16 (43%) cases. Ten of 11 (91%) C4d positive cases were DSA positive, which accounted for 27% of all cases included in the study. All these 10 cases that showed concordant C4d and DSA positivity exhibited histologic features of T cell-mediated rejection in portal tracts. Twenty (54%) cases were negative for both C4d and DSA. The remaining 7 (19%) cases showed non-concordant C4d and DSA status.

Conclusions: Concordant DSA and C4d status is demonstrated in 81% of the cases in this study. Positive DSA and positive C4d-IR are detected in 43% and 30% of posttransplant liver biopsies that show histologic features of CP. Concordant DSA and C4d positivity is demonstrated in 27% of the cases. These data suggest that antibody-mediated mechanisms are implicated in the development of CP in a subset of patients, which should be considered in the setting of refractory/persistent rejection.

1704 Hexokinase Domain-Containing Protein 1 (HKDC1) Is Overexpressed and Correlated with the Histological Progression of Nonalcoholic Fatty Liver Disease

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Background: Nonalcoholic fatty liver disease (NAFLD) presents as a spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH). NASH is progressive, but its pathogenesis remains poorly understood. HKDC1 is a recently identified hexokinase-like gene, and analysis of the amino acid sequence of HKDC1 suggests that it may function as a hexokinase. A recent genome wide study has shown genome wide association of variants in HKDC1 and gestational glucose homeostasis in human pregnancy. Genetic HKDC1 knockout mouse models have revealed that HKDC1 expression is associated with hepatic fat levels. The aim of the study is to explore HKDC1 expression in NAFLD patients in the context of disease progression.

Design: Liver tissue were collected from individuals with histologically normal livers (n= 22) and patients with NAFLD (n=26). 11 patients with NAFLD had advanced fibrosis. Immunohistochemistry for HKDC1 was performed on formalin-fixed paraffin-embedded liver sections. The cellular localization and intensity of immunostain, percentage of cells stained with HKDC1 protein were studied. Based on staining intensity, the immunostain was graded as 0 (no expression), 1 (weak expression), 2 (moderate expression) and 3 (strong expression). Staining intensity was compared across the groups using Pearson's Chi-square test. A $P<0.05$ was considered to be statistically significant.

Results: HKDC1 expression was identified in cytoplasm of human hepatocytes with a fine granular pattern. There was only a minimal expression of HKDC1 protein in normal hepatocytes. In contrast, HKDC1 protein expression was significantly increased in livers with NAFLD, which was characterized by a strong expression in steatotic hepatocytes and a positive correlation with the degree of steatosis ($r= 0.74$, $p<0.001$). This increase was further accentuated in steatohepatitis with lobular inflammation and hepatocyte ballooning ($r=0.83$, $p = p<0.001$). Furthermore, there was a diffuse strong expression of HKDC1 with advanced fibrosis in the NAFLD cases.

Conclusions: This is the first study reporting an association of hepatic HKDC1 expression with NAFLD. HKDC1 was observed to be overexpressed in livers with NAFLD and its expression was positively correlated with the histological progression of this disease. Hepatic fat accumulation in NAFLD might be mediated by an increase in HKDC1 expression; therefore, HKDC1 might be a potential target for treatment of NAFLD.

1705 Combined Hepatocellular –Cholangiocarcinoma with Stem Cell Features: Pitfalls in Diagnosis

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Background: Combined hepatocellular-cholangiocarcinoma(HCC-CC)with stem cell(SC)features has 3 subtypes per WHO 2010:typical,intermediate (INT),cholangiolocellular(CLC).This has led to confusion in definition,resulting in many studies using imprecise criteria.Proper criteria are important as diagnosis of HCC-CC compared to HCC affects chemotherapy regimen and may lead to transplant denial.This study examines pitfalls in diagnosis of HCC-CC with SC features based on a case series and literature review.

Design: 28 HCC-CC were reviewed.SC features were defined(per WHO)as small tumor cells with oval hyperchromatic nuclei and high N:C. Per WHO,both HCC and CC were required in addition to SC for diagnosis.Diagnostic criteria used in 10 published studies of HCC-CC with SC were reviewed.

Results: SC features(>5%)were seen in 10(35%)HCC-CC and were prominent(>20%) in 6(21%)cases.Mix of WHO subtypes(typical,INT,CLC)was seen in all 6 cases with prominent SC. SC areas were -ve for hepatocellular markers,and variably CK7/CK19 +ve. In addition to 28 HCC-CC, there were 5 HCC(without CC)and 5 CLC(without HCC)with SC features;these were not diagnosed as HCC-CC.Review of 10 recent studies showed that 48-79% of putative HCC-CC lacked both components.

Pitfall in diagnosis	Studies (PMID#)	Comment
HCC with SC areas called HCC-CC without CC component: 85/108 (79%), total number based on review of 5 studies	23388123; 23955451;18184271; 14739102; 26969740	Should be classified as HCC with SC features
CC with SC areas called HCC-CC without HCC component: 28/58 (48%), total number based on review of 5 studies	23388123; 23955451; 26969740; 11182037; 23192764	Should be classified as CC with SC features
Scirrhous HCC called HCC-CC with SC, intermediate type: 80 cases, total number based on review of 4 studies	23388123; 23955451; 14739102; 26969740	59 (74%) are scirrhous HCC (based on provided staining pattern and images)

Conclusions: SC features are insufficient for diagnosis of HCC-CC;both HCC and CC must be present.Category of HCC(or CC)with SC features should be created for cases with 1 component having SC features.Subtypes of HCC-CC should be discontinued as >1 subtype is present in nearly all cases.Most reported INT subtype cases are scirrhous HCC with no CC area.CLC subtype without HCC is best classified as type of CC.Definition of HCC-CC with SC features has been widely misinterpreted in literature;survival and molecular data from recent studies is hence not reliable,and additional studies with precise criteria are needed.

1706 Evaluation of Peritumoral Fibrosis in Metastatic Adenocarcinoma to the Liver Using Digital Image Analysis

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Background: Pathologists often evaluate background liver in mass directed needle biopsies. Peritumoral reaction can locally increase liver fibrosis. We determined the distance from tumor at which fibrosis returns to background levels. We used digital image analysis (DIA) to objectively evaluate the extent of peritumoral fibrosis in cases of metastatic adenocarcinoma.

Design: 27 cases of liver wedge resections for metastatic adenocarcinoma were selected for further study. A representative section of tumor with adjacent liver and a section of distant uninvolved liver were stained with a Masson trichrome and digitally scanned (Hamamatsu). The percentage of area staining positively for fibrosis in 500 µm concentric intervals was compared to the uninvolved liver using a DIA program (HALO, Indica labs). Subcapsular, large portal and venular areas were excluded from the analysis. The amount of peritumoral fibrosis was considered to have returned to baseline when it was within 1% of the uninvolved parenchyma. Spearman correlation coefficients were determined.

Results: The mean age at resection for the cases was 57 (56% male) and 89% received prior treatment. 25 cases were colorectal metastases, 1 small bowel, and 1 lung. Two patients had a prior diagnosis of liver disease, but no significant background fibrosis. The median tumor was 2 cm (interquartile range (IQR) 1.1-2.85) with 30% (IQR 7.5-47.5) necrosis. The median baseline staining level was 1.3%(IQR 1.0-1.6). Within the first 500 µm of peritumoral area, there was a 4 (IQR 2-7) fold median increase in fibrosis compared to the uninvolved liver. The median distance to return to baseline fibrosis was 2 mm (IQR 1-5). 3 cases did not return to baseline at the furthest extent measurable (5.5, 8.5, and 10 mm). Percent tumor necrosis was significantly correlated with the fold increase in peritumoral fibrosis (ρ=0.48, p=0.01) and the distance at which fibrosis returned to baseline (ρ=0.52; p=0.005). Though not statistically significant, tumor size was correlated with the fold increase in peritumoral fibrosis (ρ=0.13; p=0.50) and the distance at which it returned to baseline (ρ=0.32; p=0.10).

Conclusions: While not practical for clinical use, DIA is a powerful research tool to objectively and systematically measure fibrosis in the liver. We show fibrosis is markedly increased in the peritumoral liver and our data suggest that in the setting of metastatic adenocarcinoma, fibrosis levels typically return to baseline levels 1-5 mm from the tumor. These findings need to be validated in different tumor types and in livers with background fibrosis.

1707 PD-L1 and LAG3 Expression in Hepatocellular Carcinoma Associated with HCV and Steatohepatitis

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Background: Inhibitory immune checkpoint regulators PD1/PD-L1 and LAG3 are therapeutic targets for patients with advanced melanoma and non-small cell lung cancer. Patients with hepatocellular carcinoma (HCC) ineligible for curative surgery are refractory to conventional therapies. Immunotherapy could be a promising treatment strategy for HCC patients. Defining the HCC immune microenvironment will determine if the therapeutic targets are present in tumor. We wanted to determine the expression of immune checkpoint inhibitors in HCC that arise in hepatitis C viral (HCV) infection and steatohepatitis (SH).

Design: Twenty nine (n=15 HCV and n=14 SH) surgical HCC specimens were examined. Clinical information was recorded with confirmatory histologic review. Sections of background liver and HCC were selected for immunohistochemistry for CD8, PD-L1, PD-1, and LAG3. Scanned (Aperio Scanscope) slides (20x) underwent image analysis (HALO Indica Labs) to determine the density of CD8 and PD-1 positive cells within the tumor and background liver. To assess PD-L1 expression the number of clusters (> 5 cells staining with PD-L1 in a membranous pattern) were counted. Both PD-L1 expressing hepatocytes and tumor associated macrophages were counted and the densities of positive cell clusters were scored. The percentage of LAG3 positive lymphocytes was scored in 5 low power fields. PD-L1 and LAG3 were scored by two pathologists. Student t-test is used for statistics.

Results: There were significantly fewer CD8 (14.3 (16.9) vs 43.0 (32.6)) expressing lymphocytes within the HCC than in the in background liver (P<0.001). There were significantly more LAG3 positive lymphocytes (12.8 (18.4) vs 3.0 (6.7)) and PD-L1 (8.9 (10.8) vs 0.4 (1.2)) positive clusters in HCC than in background liver (P<0.001). No difference in the percentage of PD-1 positive lymphocytes observed in HCC and the background liver (P=0.145). We found no difference in any of the markers we tested in patients with chronic HCV and SH.

Conclusions: This study shows there is expression of check point inhibitors in HCC. A correlation between check point inhibitor expression and CD8 infiltration suggests that liver cancer might suppress the immune system using the adaptive immune resistance mechanism. The underlining chronic liver disease does not appear to alter the immune microenvironment in HCC. This study suggests immune modulatory therapy may be beneficial to HCC that arises in chronic viral infection and fatty liver disease.

1708 Hepatic Epithelioid Angiomyolipoma: Immunohistochemical Pitfalls in Diagnosis

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Background: Hepatic angiomyolipoma (AML) often shows an epithelioid morphology, and the fat element may be inconspicuous or absent. The epithelioid component can mimic hepatocellular tumors like hepatocellular adenoma (HCA) or hepatocellular carcinoma (HCC). This study examines the results of commonly used stains for HCA and HCC in AML, and highlights the pitfalls in diagnosis.

Design: H&E slides were reviewed and immunohistochemistry was performed for glutamine synthetase (GS), β-catenin, and liver fatty acid-binding protein (LFABP) in 8 resected hepatic AMLs. All cases were sporadic and there was no history of tuberous sclerosis. Sequencing of exon 3 of *CTNNB1* gene (β-catenin) was done in 3 cases.

Results: Predominantly epithelioid component (>50%) was seen in 5 (63%) cases, and lack of fat in 2 (25%) cases. Foamy macrophage collections were present in 3 (38%) cases. Aggressive histologic features were often present: moderate to severe cytologic atypia (50%), mitoses (38%) and necrosis (25%). A fragmented reticulin framework similar to HCC was seen in the epithelioid component in 1 case. GS staining (>10%) was seen in the epithelioid component in 7 (88%) cases, and was diffuse (>50%) in 3 (38%) cases. LFABP staining and nuclear β-catenin staining were not seen in any case.

Stain	Result	Comment
Reticulin	Fragmented framework: 1/8	Can be mistaken for HCC
GS	Positive (>10%): 7/8 Diffuse positive (>50%): 3/8	Can be mistaken for β-catenin activated HCA, or for HCC if cytologic atypia is present
LFABP	Negative : 8/8	Can be mistaken for HNF1-alpha-inactivated HCA

Mutations in exon 3 of *CTNNB1* gene (β-catenin) were not identified in any of the 3 cases tested (2 diffuse GS, 1 patchy GS). There was no recurrence or metastasis in any case (mean follow-up 38 months).

Conclusions: Predominance of epithelioid component and inconspicuous fat is common in hepatic AML, leading to resemblance with HCA and HCC. Absence of LFABP and presence of fat can be mistaken for HNF1-alpha inactivated HCA. Diffuse GS is seen in the epithelioid component in nearly half of the cases, which can be mistaken for β-catenin activated HCA or HCC. Lack of nuclear β-catenin staining and absence of mutations in exon 3 of *CTNNB1* gene (β-catenin) indicates an alternative mechanism of GS overexpression, presumably related to sporadic *TSC1*/*TSC2* mutation (PMID:16552619). Foamy macrophage clusters are frequently present can be useful clue to the diagnosis of AML. Adverse histologic features do not predict an adverse outcome.

1709 Angiosarcoma of the Liver: Study of Morphologic Patterns and Report of Three Novel Variants

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Background: Angiosarcoma of the liver is a rare malignant neoplasm. The diagnostic challenge is often to recognize various morphologic
patterns in order to get the proper immunostains. The limited published data on histological patterns can make this particularly challenging. To provide more comprehensive data, we reviewed angiosarcomas from a larger medical referral center.

Design: All patients diagnosed with angiosarcomas of the liver between 1996 and 2016 were retrieved. The major growth patterns were classified as sinusoidal (non-mass forming) versus mass forming. The mass forming cases were subdivided into epithelioid, spindled, vasoformative, or others.

Results: The study identified 30 patients: 23 male and 7 female. The ages ranged from 34 to 89 years. Seven of thirty patients underwent resection. **Mass forming:** The tumors were mass forming in 22 cases, of which 12 showed predominantly vasoformative growth. Eight of these cases were composed of small vessels, 3 had slit-like vascular spaces and one showed a mixture of small and large vessels. One of 30 cases showed a mixture of vasoformative and non-vasoformative areas, with spindled and epithelioid morphology. Of the mass forming cases, 9 had a non-vasoformative solid growth, of which 6 cases had an epithelioid morphology, with large plump cells, abundant cytoplasm, and prominent nucleoli. Three of 9 cases with a solid growth pattern were composed primarily of spindled cells. **Non-mass forming:** Five of 30 cases were non-mass forming and showed either diffuse sinusoidal infiltration (N=3) or prominent peliotic changes (N=2). **Unique patterns:** Three unique patterns were identified. One case showed nodules of spindle cells arranged in prominent whorls in a background of loose connective tissue with abundant inflammation. A second case showed numerous nodules with an architectural pattern that resembled infantile hemangioma. The third variant showed multiple nodules of thin walled large caliber vascular proliferations, of which some showed varying degrees of dysplasia and frank angiosarcoma.

Conclusions: Most of the angiosarcomas are mass forming (2/3 of cases), a pattern that is recognizeble when vasoformative, but can mimic carcinoma when nonvasoformative (1/3 of cases). The sinusoidal patterns are challenging and are frequently missed on initial review. Finally, we describe several unreported patterns that can be can be particularly challenging to diagnose (1/10 of cases). Awareness of these classic and unique morphologic variants can help make the diagnosis of angiosarcoma.

1710 Diagnostic Utilities of Electronic Microscopy(EM) in Genetic and Metabolic Liver Diseases - A Study of 105 Cases in Chinese Population

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Background: Metabolic liver diseases represent a unique group of medical hepatology cases. A spectrum of relevant clinic-pathological characteristics aids in their differential diagnosis. However, a lot of similar clinic-pathological features among these diseases make medical hepatopathology a challenging subspecialty. To help clinical decision-making, electronic microscopical examination provide unique value. Though EM studies of genetic and metabolic liver diseases have been documented in the western countries, the EM utilities in this group of liver diseases have not systematically reported in Chinese population.

Design: This study coordinated ultrastructural results with traditional histology, relevant clinical as well as laboratory tests in diagnosis of genetic and metabolic liver diseases, in order to sub-classify diseases and to guide more efficient use of this special tool in clinical practice, particularly for Chinese population.

Results: Our department receives medical liver cases across China through our network of 31 provincial laboratories. From Jan 1, 2015 to Sept 15, 2016, 105 consecutive cases were enrolled into this study. Four cases were eliminated due to lack or suboptimal tissue for electronic microscopy. Among the remaining 101 eligible cases, 60 were Glycogen storage diseases (GSD), 24 Hereditary hyperbilirubinaemia(HH), 6 hepatic amyloidosis(HA), 4 diseases(WD), 4 primary hemochromatosis(PH), 2 porphyria cutanea tarda(PCT), and 1 mucopolysaccharidose(MPS).

Based on the clinicopathological parameters, 40 GSDs were indicated, while the other 20 GSDs were missed, which were only diagnozed by EM. The results were summarized in Table 1 below.

	EM confirmation/initial diagnosis	Missed by Clinicopathological parameters
GSD: 40	60/20	20
HH: 6	24/18	6
HA: 6	6/6	0
WD: 2	4/2	2
PH: 4	4/4	0
PCT: 2	2/2	0
MPS: 1	1	0

Conclusions: Electronic microscopy plays a role in diagnosis of genetic metabolic liver diseases. It is particularly helpful in evaluation of GSD and HH of Chinese population.

1711 Diagnostic Value of Clusterin Immunostaining in Hepatocellular Carcinoma: A Comparison with pCEA and CD10

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Background: Histopathologic distinction of hepatocellular carcinoma (HCC) from benign hepatocellular mass lesions and non-hepatocellular malignancies is clinically important but can be difficult. Immunostains for carcinoembryonic antigen (using polyclonal antibodies; pCEA) and CD10 can help determine hepatocellular origin by demonstrating a canalicular staining pattern, but these immunomarkers do not appear to discriminate between benign and malignant hepatocellular lesions. We have previously observed an enhanced canalicular staining pattern for clusterin (CLU) that appears unique to HCC. The current study further investigated its diagnostic value by comparing with pCEA and CD10.

Design: Forty-seven surgically resected HCCs were included in this study. Tissue sections were immunohistochemically stained for CLU, pCEA and CD10. Immunostained slides were analyzed for canalicular staining patterns. The staining intensity was compared between HCC and nonneoplastic liver tissue to determine if the canalicular immunoreactivity in HCC is stronger (enhanced), equivalent or weaker. The results were compared among CLU, pCEA and CD10.

Results: Of 47 HCCs, 46 were well or moderately differentiated. One case had poorly differentiated areas. None of the cases was treated with neoadjuvant chemotherapy or embolization. Canalicular CLU staining was observed in 42 (89%) HCCs, which was strong in 26 (62%), intermediate in 14 (33%) and weak in 2 (5%) cases. In 63% of cases with nonneoplastic liver tissue present on the same tissue sections, CLU immunoreactivity was enhanced in HCC in comparison with nonneoplastic tissue. Positive canalicular staining for pCEA and CD10 was observed in 91% and 66% of HCCs, but enhanced expression in HCC was demonstrated in only 14% and 17% of cases ($p<0.001$), respectively. The majority of pCEA and CD10 positive cases showed equivalent staining intensity between HCC and nonneoplastic liver tissue.

Conclusions: An enhanced canalicular CLU staining pattern is observed in two-thirds of HCCs. This unique staining characteristic is superior to that of pCEA and CD10 in that it helps distinguish HCC not only from non-HCC malignancies but also from benign liver tissue.

1712 Glypican 3 as a Serum Marker for Hepatoblastoma

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Background: Hepatoblastoma (HB) is the most common primary malignant liver tumor in children. The conventional serum marker for HB, alpha-fetoprotein (AFP), has its limitations. Novel serum markers for HB need to be explored. Glypican 3 (GPC3) has been reported to be an excellent histological immunomarker for both HB and hepatocellular carcinoma (HCC) and a promising serum marker for HCC. However, the clinical value of serum GPC3 in HB patients is unknown.

Design: Between June 2013 and July 2016, a total of 184 serum samples were collected. Of these, 134 were from 32 HB patients at different treatment stages (22 from pretreatment, 70 during treatment and 42 in clinical remission), 30 from age-matched patients with benign hepatobiliary disorders (BHD) and 20 from age-matched normal controls (NC). Each sample was measured for GPC3 by ELISA and AFP by immunometric assay.

Results: The median serum GPC3 levels were 1.93 ng/ml (range, 0 - 31.19) in HB pretreatment group, 1.74 ng/ml (range, 0 - 25.95) in BHD group, 0.59 ng/ml (range, 0 - 6.20) in NC group, 0.94 ng/ml (range, 0 - 17.56) in HB during treatment group and 0.57 ng/ml (range, 0 - 11.54) in HB remission group. The GPC3 levels in HB pretreatment group were significantly higher than those in NC group ($p=0.0029$) and HB remission group ($p= 0.006$) but not statistically different from those in BHD group ($p=0.6235$) and HB during treatment group ($p=0.0598$). In contrast, AFP levels in HB pretreatment group were significantly higher than those in both BHD and NC groups ($p<0.0001$ for both). And there was a significant difference in AFP levels among HB pretreatment, during treatment and remission groups ($p<0.0001$ for all two group comparisons). The areas under the receiver operating curve (AUROC) value, sensitivity and specificity of GPC3 for HB pretreatment group versus all controls were 0.63 (95% CI: 0.49 to 0.77), 54.5 % and 66%, respectively, at a cutoff of 1.74 ng/mL, all significantly lower than those of AFP (0.91 (95% CI: 0.84 to 0.97), 95.5% and 76.0%, respectively) at a cutoff of 1130 ng/ml). Neither serum GPC3 nor AFP levels were associated with prognostic parameters such as age, tumor size, tumor stage, lymphovascular invasion or metastasis. There was no significant correlation between serum levels of GPC3 and AFP in patients with HB ($p=0.422$).

Conclusions: GPC3 is inferior to AFP as a serum marker for HB.

1713 Liver Injury Induced by PD-1 Inhibitor Nivolumab: Report of 3 Cases with Biopsy Findings

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Background: Nivolumab, a humanized IgG4 anti-PD-1 monoclonal antibody that activates T cell-mediated tumor suppression, is being evaluated for the treatment of various human cancers including advanced hepatocellular carcinoma (HCC). Clinical trials have shown that nivolumab therapy is associated with serum aminotransferase elevations in >10% of patients. Histopathologic features of nivolumab induced liver injury have not been well described. It has been associated with rejection in kidney transplant recipients, but its effects on transplanted livers have not been reported.

Design: Liver biopsies from 3 patients who received nivolumab therapy were evaluated. Detailed clinical history and follow-up data were obtained.

Results: All 3 patients were male aged 14, 20 and 59 years, respectively. Both younger patients were liver transplant recipients for fibrolamellar HCC, who developed lung metastases at 12 and 40 months after transplantation. The third patient had recurrent Hodgkin lymphoma after allogeneic stem cell transplantation. Marked elevations of serum transaminase (AST 8-26 times ULN; ALT 6-17 times ULN), alkaline phosphatase (3.3-5 times ULN) and bilirubin (4-11 times ULN) levels were observed in all 3 patients within 1-3 weeks after initiation of nivolumab therapy (3 mg/kg), accompanied by fever, headache and abdominal pain. Biopsies from both transplanted livers showed ductopenia, patchy hepatocyte necrosis and cholestasis. One biopsy also showed moderate portal inflammatory cell infiltrates and central perivenulitis, while the other showed only minimal portal inflammation. Portal vein endotheliitis was not evident. Both patients had high titers of donor-specific antibodies but had negative C4d immunostains. Liver biopsy from the patient with Hodgkin lymphoma showed mild portal lymphocytic inflammation, bile duct injury, lobular disarray with mild spotty necroinflammation, and cholestasis. Nivolumab therapy was terminated in all 3 patients who were also treated with steroids. On follow-up, both liver transplant recipients died of liver failure 4 weeks later. The remaining patient died of *Achromobacter* pneumonia and invasive aspergillosis.

Conclusions: Nivolumab induces severe liver injury presumably via immune-mediated mechanisms. In the transplant setting, nivolumab may exacerbate allograft dysfunction by promoting both T cell- and antibody-mediated rejection.

1714 Utility of 5hmC Immunohistochemical Expression in the Diagnosis of Hepatocellular Carcinoma

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Background: Dysregulation of epigenetic pathways, including mutations to TET proteins, has been previously implicated in the pathogenesis of hepatocellular carcinoma. A potential marker of disruption to these epigenetic pathways is 5-hydroxymethylcytosine (5hmC), an intermediary in the DNA methylation pathway. Loss of 5hmC has been shown to be an epigenetic hallmark of melanoma as a result of TET2 dysfunction. 5hmC deficiency has also been shown to be correlated with epigenetic gene mutations in acute myeloid leukemia. The aim of our study was to evaluate the immunohistochemical expression of 5hmC in hepatocellular carcinoma (HCC) compared to hepatic adenoma (HA) and benign hepatocytes.

Design: Thirty-three liver resections or core biopsies from our institute were enrolled under an IRB-approved study. IHC staining for 5hmC was performed on formalin-fixed and paraffin-embedded (FFPE) tissue sections. Nuclear staining of 5hmC was scored by recording the percentage of cells stained for 5hmC (0=no staining; 1=1-10%;

2=11-50%; 3=51-100%) and intensity of staining (0-3+). A 5hmC score was calculated by multiplying the percentage and intensity scores (range 0-9), and a score of <3 was considered deficient for 5hmC.
Results: Normal and cirrhotic liver parenchyma showed predominantly intact expression of 5hmC, with 5hmC scores ranging from 3-9. In some cases, staining was weak (1+) but present in the majority of cells. In contrast, expression of 5hmC was deficient in 93% (25/27) of HCC cases. The intensity and percentage of 5hmC expression in HCC were dramatically reduced compared to surrounding benign hepatocytes or cirrhotic nodules. No significant difference in 5hmC expression was seen between HCC tumor grades (Chi-Square test, p=0.19). 83% (5/6) cases of HA exhibited intact 5hmC expression, with most cases showing slightly reduced 5hmC expression compared to neighboring benign hepatocytes.
Conclusions: Nearly all cases of HCC in our cohort were deficient for 5hmC expression, compared to intact 5hmC expression in normal/cirrhotic surrounding hepatocytes and HA cases. 5hmC could serve as an important diagnostic tool in the diagnosis of HCC, and suggests an underlying role for epigenetic dysfunction in liver tumorigenesis.

Neuropathology and Ophthalmic Pathology

1715 BAP1 Mutations in Rhabdoid Meningiomas

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Background: Patients with meningiomas have widely divergent clinical courses. Some entirely recover following surgery alone while others have relentless tumor recurrences which can lead to death. This clinical conundrum is exemplified by rhabdoid meningiomas which are designated in the World Health Organization (WHO) Classification of Tumors as the highest grade of meningioma, despite only a subset actually following a highly aggressive clinical course. Patient management decisions are further exacerbated by high rates of interobserver variability amongst diagnosticians, biased against missing possibly aggressive tumors. Objective molecular determinants are therefore needed to guide clinical decision-making and to prevent both over-and under- treatment.
Design: We performed sequencing of cancer related genes in 26 meningioma with rhabdoid features and evaluated BAP1 protein expression by immunohistochemistry in 336 meningiomas. We assessed outcomes, germline status and family history in patients with BAP1-negative rhabdoid meningiomas.
Results: The tumor suppressor gene BAP1 (BRCA1–associated protein 1) is inactivated in high-grade, rhabdoid meningiomas. Patients with BAP1-negative rhabdoid meningiomas had reduced time to recurrence compared with patients with BAP1-retained rhabdoid meningiomas (Kaplan Meier analysis, 26 months vs 116 months, $p < 0.001$). A subset of patients harbored germline BAP1 mutations, indicating that rhabdoid meningiomas can be a harbinger of the BAP1 cancer predisposition syndrome.
Conclusions: We define a subset of aggressive rhabdoid meningiomas that can be recognized using routine laboratory tests. We implicate ubiquitin deregulation in the pathogenesis of these high-grade malignancies. In addition, we show that familial and sporadic BAP1-mutated rhabdoid meningiomas are clinically aggressive, requiring intensive clinical management.

1716 Diverse Patterns of Involvement in Intraocular Lymphoma: Learning from Four Enucleation Specimens

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Background: Intraocular lymphomas are rare and often pose challenges both clinical and diagnostically. The majority of cases are diagnosed on vitrectomy specimens; enucleation is seldom performed for diagnostic purposes. Most enucleated specimens are submitted as *post-treatment blind painful eye*. Herein,we describe the diverse patterns of involvement in enucleation specimens involved by intraocular lymphomas, 2 primary vitreoretinal lymphomas naïve of treatment and 2 systemic lymphomas with intraocular involvement.
Design: Computer search of Anatomic Pathology files (January 1994 to November 2015) revealed 4 enucleation specimens with intraocular lymphoma. Electronic medical records were reviewed for demographics, clinical findings, laboratory and radiographic studies results, and indication for enucleation. The slides in all cases were reviewed.
Results: Two cases were large B-cell vitreoretinal lymphomas, naïve of treatment, in 80 and 75 year old women treated for uveitis with no success. Both specimens showed extensive retinal and sub-retinal infiltrates of large atypical B-lymphocytes (positive for CD20, Bcl6, MUM1 and MYC, among several markers tested). Rare neoplastic cells were present in the vitreous, and the choroid had only reactive infiltrate of small lymphocytes (CD3 positive T-cells). One case showed MYD88L265P mutation by PCR. Remaining two cases were on patients with systemic lymphoproliferative disorders. The first, a 65 year old man with underlying CLL, complaining of floaters, was diagnosed with large B-cell lymphoma on vitrectomy, consistent with intra ocular Richter's transformation. The enucleated eye showed treatment effect and residual lymphoma involving retina and choroid. The last case, a 69 year old woman with stage III diffuse large B-cell lymphoma in remission, developed blurred vision on left eye, was treated for panuveitis without improvement, and later diagnosed on choroidal biopsy with ocular involvement by large B-cell lymphoma. The enucleated specimen showed focal residual lymphoma located in the choroid.
Conclusions: Different patterns of ocular tissue involvement were observed in these four cases when we compared primary and secondary intraocular lymphomas. The paucity of vitreous tumor cells in the 2 cases of vitreoretinal lymphoma supports the diagnostic challenge one faces in some vitrectomy specimens. In addition, one of our cases exemplifies the unusual occurrence of Richter's transformation in the eye.

1717 Differential Exon Expression Analysis in Glioblastomas Multiforme by Quantitative Statistics

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Background: Glioblastoma multiforme (GBM) is afflicted with dismal prognosis despite the fact that major aspects of its molecular genetics have been comprehensively investigated. Although differential exon usage is known to mediate distinct biological functions, until now only a few expression studies have investigated exon splicing events in GBM leaving a gap in the knowledge of the molecular genetics of this disease.
Design: We conducted a quantitative statistical analysis on microarray expression datasets which are curated by the Gene Expression Omnibus (GEO) and which used HuGene 1.0 ST microarrays for analysis. This type of whole transcript microarray interrogates with a set of 764,885 probes 36.079 annotated reference sequences representing 253002 exons (NCBI build 36). The data sets consisted of 71 GBM that were compared to 14 non-GBM gliomas comprising astrocytoma, oligoastrocytoma, and oligodendroglioma cases. A p-value with a false discovery rate (FDR) < 0.05 and an exon-based fold change > 2 (> 10 for a comprehensive short list) were used as a threshold of significance for differential expression (Transcriptome Analysis Console v3.1, Affymetrix, Santa Clara, CA).
Results: We identified in our quantitative analysis a number of genes which contained one or more exons that were both, differentially expressed between GBM and non-GBM gliomas and in the GBM group comparably lower expressed than other exons from the same gene. Among the identified genes were semaphorin 3D (*SEMA3D*), mohawk homeobox (*MKX*), RALBP1 associated Eps domain containing 2 (*REPS2*), and cholecystokinin (*CCK*). Different splice variants for these genes are described. In addition, a number of identified genes are likely to harbor exons that are differentially expressed in non-GBM gliomas or differentially expressed in both, GBM and non-GBM gliomas.
Conclusions: Combining publicly available expression datasets and using for statistical comparison non-GBM gliomas which are histologically related to GBM, we were able to identify a number of candidate genes that contain differential expressed exons. Of notice, SEMA3D which is a secreted semaphorin receptor binding protein is known to mediate breakdown and paralysis of neuronal growth cones and to inhibit glioblastoma cell proliferation *in vivo*. In further studies we have to sustain our *in silico* findings in clinical samples and functional assays.

1718 Focal Cortical Dysplasia-Associated Low-Grade Glioma: Expending the BRAF Mutations Concept

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Background: It has been shown that focal cortical dysplasia (FCD) and gangliogliomas originate from a common precursor that undergoes abnormal glioneuronal development. Furthermore, it has been recently demonstrated the presence of *BRAF* mutations in low-grade epilepsy-associated tumors. Here we demonstrate that *BRAF* mutations are present, not only in the tumor, but also in the adjacent dysplastic neurons.
Design: Electronic records were searched for pediatric patients with FCD and associated low-grade glioma in the last 10 years. In all patients, slides were reviewed and representative sections were selected. BRAF molecular status was evaluated in the low-grade glioma, in the FCD, and in the adjacent normal cerebral cortex. For this purpose, DNA was extracted from paraffin-embedded tissue blocks using laser capture microdissection. In addition to the associated tumor, normal and dysplastic neurons were microdissected from normal and dysplastic cerebral cortex, respectively.
Results: The search resulted in 7 cases of concomitant FCD and low-grade gliomas. The gliomas were as follows: 2 diffuse astrocytoma (DA) WHO grade II, 3 ganglioglioma (GG) WHO Grade I, and 2 pleomorphic xanthoastrocytoma (PXA) WHO grade II. The patients consisted of 5 males and 2 females (Age range: 5-13.5 years; average: 8.6 years). Six tumors were in the temporal lobe and one (PXA) in the frontal lobe. *BRAF* mutation (V600E) was present in two DA and one PXA. In both cases, *BRAF* mutations were concomitantly present in the associated FCD. In one DA and one GG, *BRAF* mutation was present only in the tumor while the adjacent dysplastic neurons and normal neurons showed no mutation. No *BRAF* mutation was noted in the normal cerebral cortex in all 7 patients. FCD consisted of 3 cases of type IIa and 4 cases of type IIIb.
Conclusions: Our results suggest that FCD and FCD-associated glioma represent a spectrum in the "focal cortical development malformation" concept. Larger studies are required in order to understand the significance of *BRAF* mutations in FCD as well as to investigate the role of *BRAF* mutations in epilepsy-associated tumors and FCD and their role as prognostic tumor behavior marker.

1719 Single Nucleotide Polymorphism Microarray Analysis of Pineocytomas

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Background: Pineocytomas are well-differentiated tumors of the central nervous system that arise from the pineal gland parenchyma. Their rarity, bland histologic features, and potential to demonstrate cystic architecture, which may render them indistinguishable from non-neoplastic pineal cysts via imaging, all contribute to the persistent diagnostic