278 Characterization of cultured cholangiocytes isolated from livers of patients with primary sclerosing cholangitis

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Introduction: Primary sclerosing cholangitis (PSC) is a chronic, idiopathic, incurable cholangiopathy in which the pathogenesis remains obscure, in part because of the lack of appropriate experimental models. Here, through cellular, molecular, and next-generation sequencing (NGS) methods, we describe the development of a PSC patient-derived cholangiocyte cell line and characterization of the phenotypic and signaling features. Methods: We isolated cholangiocytes from stage 4 PSC patient liver explants by dissection, differential filtration, and immune-magnetic bead separation. We maintained cholangiocytes in culture and assessed for: i) cholangiocyte, cell adhesion, and inflammatory markers; ii) proliferation rate; iii) transepithelial electrical resistance (TEER); iv) cellular senescence; and v) transcriptomic profiles by NGS. We used two well-established normal human cholangiocyte cell lines (H69 and NHCH) for comparison. Results: Isolated PSC cells expressed cholangiocyte (e.g. cytokeratin 7 and 19) and epithelial cell adhesion markers (EPCAM, ICAM) and were negative for hepatocyte and myofibroblast markers (albumin, α-actin). Proliferation rate was lower for PSC compared to normal cholangiocytes (4 vs. 2 days, respectively, p<0.01). Maximum TEER was also lower in PSC compared to normal cholangiocytes (100 vs. 145 Ωcm2, p<0.05). IL-6 and IL-8 (protein and mRNA) were both increased compared to NHCHs and H69s (all p<0.01). The proportion of cholangiocytes staining positive for senescence-associated β-galactosidase was markedly higher in PSC cholangiocytes compared to NHCHs (48% vs. 5%, p<0.01). Lastly, NGS confirmed cholangiocyte marker expression in isolated PSC cholangiocytes and extended our findings that pro-inflammatory and senescence-associated markers are increased in PSC compared to normal cholangiocytes. Conclusions: We have demonstrated that high-purity cholangiocytes can be isolated from human PSC liver and grown in primary culture. Isolated PSC cholangiocytes exhibit a phenotype that may reflect their in vivo contribution to disease and serve as a vital tool for in vitro investigation of biliary pathobiology and identification of new therapeutic targets in PSC.

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quality of life. However, fatigue in PBC is not related to measures of disease severity. Despite its impact on patients, the cause of fatigue in PBC remains poorly understood. Previous functional magnetic resonance imaging (fMRI) studies have demonstrated an association between putamen (part of the basal ganglia) activity and fatigue in a number of non- hepatic disorders. Therefore, we used resting-state fMRI (ie. in the absence of a task) to determine if functional connections with the putamen are altered in PBC patients in association with fatigue scores. Methods: Ten PBC patients (none with advanced liver fibrosis) and ten sex- and age-matched healthy controls underwent a resting-state fMRI scan. Brain maps of functional connection strength with the putamen were generated using time series analysis. These maps were compared between groups, using each patient’s Fatigue Severity Scale (FSS) score as a covariate. Results: Compared to healthy controls, PBC patients exhibited reduced functional connection strength with the right thalamus (receives sensory input from the body), the left globus pallidus (sends inhibitory input to the motor system), and areas of the brain involved in emotional processing (including the right anterior cingulate cortex and bilateral caudate). In addition, PBC patients exhibited reduced functional connection strength with bilateral premotor cortices, involved in refining motor movements and providing input to the thalamus. Greater FSS scores were associated with decreased functional connection strength with the right primary somatosensory cortex (receives input from the thalamus) and left hippocampus (involved in memory)(Figure 1). Conclusions: Our results suggest that PBC patients exhibit reduced functional brain connectivity with areas of the basal ganglia, which have been implicated in fatigue. These data also suggest that PBC impacts the motor network of the brain, which could contribute to clinical manifestations of fatigue. Moreover, patients that report higher levels of fatigue exhibit a further reduction of functional connection strength between the putamen and the right superior frontal gyrus, suggesting that symptom severity can manifest as measurable changes in the functional organization of the brain.

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Sub-stratification of hepatocellular carcinoma risk in men with primary biliary cirrhosis: results of an international multicenter study

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Background: Hepatocellular carcinoma (HCC) is an infrequent yet critical event in primary biliary cirrhosis (PBC) and development is heavily influenced by patient gender. However, it remains unclear whether HCC risk can be further stratified in men, a population deemed inherently high-risk. Methods: Individual patient-data was collected from over 15 North American and European liver centres, spanning >40-years observation period. Risk-factor analysis was performed using Cox proportional hazard regression models and Kaplan-Meier estimates (SPSSv21). Results: Across a cohort of 4565 patients with confirmed PBC (median follow-up 7.1 years), 123 cases of HCC were identified. Men were more likely to develop HCC than women (incidence rate: 6.7 vs. 2.6 cases per 1,000 patient years; HR: 2.91, 1.9-4.8 p<0.0001), and this difference retained significance when restricting the analysis to males and females with advanced disease at PBC diagnosis (HR 2.9, 95% CI 1.60-5.32, p<0.001). However, significant differences between genders were no longer apparent when the incidence was compared in patients with early-stage PBC (p=0.49). The proportion of PBC patients receiving ursodeoxycholic acid (UDCA) was similar between men and women (84% versus 85%, respectively; p=0.75); however, on stratifying for biochemical response (Paris-I) the highest HCC risk was observed in non-responding male patients, and significantly greater than male-responders and female non-responders (overall log-rank p<0.001; Figure 1). Conclusion: Male gender is a significant risk factor for development of HCC in PBC although effective risk stratification can be furthered by assessment of disease stage and application of biochemical response criteria.
Figure 1: HCC incidence stratified according to gender and biochemical disease stage

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A Mouse Model of Fibrous Cholangiopathy

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Background and Aims: Primary sclerosing cholangitis (PSC) is an enigmatic, progressive cholestatic liver disease characterized by a fibrous cholangiopathy of large and small bile ducts. Treatment options for PSC are limited and lack robust efficacy. Both bile acid toxicity and death receptor signaling have been implicated in the pathogenesis of PSC. (Fickert et al., Am J Pathol. 2006; Takeda et al., Proc Natl Acad Sci U S A. 2008). Because death receptor signaling is modulated by cellular inhibitor of apoptosis (cIAP) proteins, we explored the role of cIAP-1 and -2 in the pathogenesis of PSC. Methods: Human PSC (N=20, stage IV) and nonalcoholic steatohepatitis liver sections (NASH) (N=20) were examined by immunohistochemistry (IHC). Mice liver sections were examined by IHC, Sirius red staining, and TUNEL assay. Mouse cholangiograms were obtained by injecting a radio opaque liquid silicone polymer containing lead chromate into the biliary system followed by microCT with 3-D reconstruction. Results: Expression of cIAP-1 and -2 proteins in interlobular bile ducts was markedly reduced in human PSC liver sections compared to NASH. To ascertain if cIAP-1 and -2 elimination was sufficient to induce PSC changes, C57Bl/6 mice fed a 0.2% diet of the hydrophobic bile acid deoxycholate (DCA) were treated with and without AT-406 (oral gavage, 100 mg/kg/d for 14 days), a SMAC mimic which induces rapid cellular elimination of cIAP proteins. Mice receiving both AT-406 plus DCA displayed a non-obliterator, fibrous cholangiopathy of the interlobular bile ducts as assessed by Sirius red staining, which was not observed with DCA alone (2.5-fold increase, p<0.01). Significant apoptosis within the bile ducts, as evidenced by a 4-fold increase in TUNEL positive cells, also occurred with AT-406 plus DCA compared to DCA alone (p<0.001). Cholangiography of the AT-406 plus DCA treated mice demonstrated irregularity of segmental bile ducts and pruning of the biliary tree. However, liver function tests revealed no elevation of alkaline phosphatase or bilirubin. Finally, AT-406 treatment of a human cholangiocyte cell line in vitro resulted in extensive elimination of cIAP-1 and -2 with caspase-dependent cell death consistent with activation of a death receptor signaling pathway. In conclusion, we have developed a murine model of non-obliterator, fibrous cholangiopathy with cholangiographic alterations of segmental and peripheral bile ducts. These data suggest new cytoprotective therapeutic strategies for PSC (i.e., caspase inhibitors).

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Increased BMI is associated rapid progression of fibrosis in Primary Sclerosing Cholangitis (PSC)

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Background: Obesity has become a global epidemic and has led to large increases in non-alcoholic fatty liver disease (NAFLD). In parallel to the general population, we have also detected an increase incidence in the mean body mass index (BMI) in individuals with Primary Sclerosing Cholangitis (PSC). Increased BMI in Primary Biliary Cirrhosis (PBC) is associated with more advanced fibrosis but the effect of BMI in PSC is unknown. Aim: To examine the effect of BMI on fibrosis stage and progression in PSC. Methods: 291 PSC patients were recruited from the Calgary PSC cohort and stratified according to initial BMI at presentation. BMI <25 as normal, 25-30 as overweight and >30 kg/m2 as obese. Fibrosis stage were measured at least once every 12 months by transient elastography using Fibroscan® and classified as F0 to F4 fibrosis. We examined the fibrosis stage at presentation and the time in months of progression to the next fibrosis stage. Data from 1368 patient years of follow up were assessed. Patients with existing cirrhosis at their first presentation or less than 1 year of follow up data were excluded. Results: 247 cases were eligible for the study. 176 individuals had a normal body weight (BMI <25), 57 were overweight (BMI 25-30) and 14 obese (BMI >30). Mean times of progression to the next fibrosis stage were 51 months, 47 months and 13 months for normal body weight, overweight and obese PSC patients respectively. In addition, obese PSC patients were associated with a more advanced fibrosis stage at presentation compared to normal or overweight cases. Conclusion: Significant proportions of patients with PSC can be classified as overweight or obese. Obesity (BMI 30 kg/m2) in PSC is associated with significantly more advanced fibrosis at presentation and more rapid fibrosis progression as measured by non-invasive transient elastography.

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284 Activation of mDCs and CD8+ T cells is associated with PSC-like cholangiopathy induced by small bowel bacterial overgrowth in a mouse model of autoimmune biliary disease

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BACKGROUND: The pathogenesis of primary sclerosing cholangitis (PSC) is largely unknown due to lack of an ideal animal model. The association of PSC with inflammatory bowel disease suggests a critical role of gut-derived factors in its pathogenesis. Our aim was to investigate the role of small bowel bacterial overgrowth (SBBO) in the development of PSC-like cholangiopathy. METHODS: We surgically created a jejunal self-filling blind loop (SBFL) to induce SBBO in a genetically susceptible mouse strain (NOD.B6Abd3), developed by introgression of a 1-Mb non-MHC insulin-dependent diabetes locus from B6 onto NOD background. Control mice underwent laparotomy (sham). Bacterial 16S rRNA gene sequencing was used to analyze bacterial populations of jejunal lumen content. H/E and Trichrome staining were used to assess hepatic inflammation and fibrosis. Flow cytometry was utilized to assess liver immune cell profiles. Chemokine expression was assessed by ELISA (serum) and by RT-PCR (liver tissue). RESULTS: Creation of SBFL led to dramatic increase in bacterial counts in jejunal lumen content, compared to sham mice. Gram-positive bacterial families Lactobacillales and Clostridiales and Gram-negative bacterial family Porphyromonadaceae were significantly less abundant in SBFL mice. However, Gram-negative bacterial families Enterobacteriaceae and Bacteroidaceae and phylum Verrucomicrobia were significantly more abundant in SBFL. Trichrome staining of liver sections revealed characteristic PSC-like lesions in 40% of SBFL mice, consisting of intrahepatic periductal fibrosis, compared to 0% of sham mice. CD11c+CD11b+PDCA1− myeloid dendritic cells (mDCs) were significantly increased in SBFL livers with PSC-like lesions (SBFL-PDF) compared to SBFL livers without PSC-like lesions (SBFL-NON-PDF). Although the expression of co-stimulatory markers CD80 and CD86 in hepatic mDCs did not show significant difference between SBFL-PDF and SBFL-NON-PDF mice, MHCI expression was significantly increased and MHCII expression was significantly decreased in hepatic mDCs in SBFL-PDF mice. Compared to SBFL-NON-PDF and sham mice, SBFL-PDF mice had significantly increased CD8+CD44+ T cells and CCL3 and CCL4 mRNA levels in the liver, and significantly increased CCL3 and CCL4 in serum. CONCLUSIONS: Our results suggest that creation of SBFL induced quantitative and qualitative changes in gut microbiota, contributing to the development of PSC-like lesions in NOD.B6Abd3 mice. The development of PSC-like lesions in NOD.B6Abd3 may be triggered by the activation and expansion of liver mDCs, which in turn recruit activated CD8+ T cells via T cell chemoattractant chemokines CCL3 and CCL4.

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285 Changes of the hepatic transcriptome in human extrahepatic cholestasis

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Background Bile salt (BS) toxicity plays an important role in cholestatic liver injury. Adaptive mechanisms are operational to reduce hepatic toxicity and promote urinary elimination of BS in cholestasis. Following up on the observation that ectopic FGF19 expression in the human cholestatic liver comprises an adaptive strategy to reduce BS synthesis (Hepatology 49:1228), we now explore the human hepatic transcriptome to gain further insight into molecular networks affected by cholestasis. Methods Total RNA was isolated from liver biopsies of patients with pancreatic tail cancer or benign liver tumors without cholestasis (controls, n=9), patients with cholestasis due to peripancreatic malignancies (cholestatic, n=9), and initially jaundiced patients with peripancreatic malignancies receiving pre-operative biliary drainage [drained, n=16]. mRNA and miRNA expression profiles were determined using Agilent arrays. Results Median total BS and bilirubin level was 194 and 186 μmol/L, resp., in cholestatic patients, with notable elevation of cholestatic injury markers [GGT 1055U/L, AP 540U/L] and transaminases [AST 232U/L, ALT 388U/L]. In patients receiving pre-operative biliary drainage total BS, bilirubin and transaminases were within the normal range. Despite resolution of cholestasis, GGT (281U/L) and AP (190U/L) remained elevated in this group. Haptic mRNA/miRNA profiles were generated with 17581 mRNAs and 504 miRNAs meeting array QC criteria. The transcriptome of control and drained groups was largely similar with only a single differentially expressed (DE) mRNA apparent (criteria: fold change >1.5 and adjusted P value <0.05). Cholestasis resulted in pronounced changes of the transcriptional landscape when compared with control (1353 DE mRNAs, 47 DE miRNAs) and drained (111 DE mRNAs, 2 DE miRNAs) groups. Overrepresentation analysis indicated a multitude of pathways affected by cholestatic conditions including ECM organization, regulation of actin cytoskeleton and bionttransformation. Alterations pertaining to BS homeostasis included downregulation of BS synthesis (CYP7A1), repression of BS uptake (SLCO1B1/3) and induction of basolateral efflux transporters (SLC51A/B) in cholestatic liver. Conclusions Extrahepatic cholestasis elicits large scale alterations in hepatic mRNA and miRNA expression. A notable difference in the number of DE mRNAs/miRNAs was apparent when comparing cholestatic with control and drained groups, with the latter two having similar serum biochemistry and identical mRNA/ miRNA profiles. Follow-up studies are required to assess the interaction between miRNA and mRNA networks and the role of the identified pathways in cholestatic liver injury.

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High serum levels and liver expression of sclerostin in patients with primary biliary cirrhosis. Association with markers of bone remodeling and severity of cholangitis

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Background and aims: Sclerostin, an inhibitor of the Wnt pathway, is involved in the regulation of osteosteblastogenesis and its role in the development of bone disease in primary biliary cirrhosis (PBC), a disease characterized by low bone formation, is unknown. Therefore, we have assessed the circulating levels and the liver gene and protein expression of sclerostin in this cholestatic disease. Methods: Serum sclerostin levels were measured in 83 women with PBC (mean age: 60 ± 12 years) and 101 control women of the same age. Lumbar and femoral bone mineral density (BMD) as well as parameters of mineral metabolism and bone remodeling (Ca/P, PTH, 25OHD, PINP, bone ALP, sCTX, NTX and osteocalcin) were measured. Moreover, sclerostin gene expression in the liver was assessed in samples of liver tissue taken by biopsy in 11 PBC patients and 5 healthy controls by real time PCR, and presence and distribution of sclerostin was evaluated in liver slices from 11 patients by immunohistochemistry. The presence and severity of histologic lesions were assessed semiquantitatively in the same liver samples. Results: Seventy-seven percent of patients had low BMD (22% osteoporosis and 55% osteopenia). PBC patients had higher sclerostin levels than controls (76.7±38.6 vs. 32.5±14.7 pmol/L, p<0.001). Serum sclerostin correlated inversely with markers of bone formation PINP (p=0.05) and osteocalcin (p=0.03), and bone resorption, NTX (p=0.01) and sCTX (p=0.03). Sclerostin mRNA in the liver was overexpressed as compared with control samples (2.7±0.3 fold vs healthy liver). Sclerostin was detected by immunohistochemistry in 7 of the 11 liver samples but not in controls, and mainly located in the bile ducts. Sclerostin was directly associated with the severity of cholangitis (p=0.02) and indirectly with the degree of lobular inflammation (p=0.03), but not with the degree of fibrosis neither with the Ludwig’s histologic stage. Sclerostin mRNA expression was higher in samples positive by immunohistochemistry (2.9 ±0.4 vs 2.5 ± 0.3, p; n.s.), and particularly in those with lobular granuloma (3.6±0.6 vs 2.4±0.2, p=0.02). Conclusion: The increased expression of sclerostin in the liver and the association with histologic cholangitis may explain the high serum levels of this protein in patients with primary biliary cirrhosis, thus suggesting that sclerostin influences the decreased bone formation in this cholestatic disease.

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Contribution of polymorphic LINE-1 retrotransposon insertion in SLCO1B3 gene to susceptibility to drug-induced cholestasis

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[Background and Aim] Drug-induced cholestasis is a rare, but sometimes, fatal disease. Underlying mechanisms remain unknown. Organic anion transporting polypeptides OATP1B1 (gene: SLCO1B1) and OATP1B3 (gene: SLCO1B3) were identified as responsible for Rotor syndrome. We have recently demonstrated that six Japanese patients with Rotor syndrome were homozygous for the insertion of LINE-1 (L1) retrotransposon in intron 5 of SLCO1B3 and for c.1738C>T (p.R580X) nonsense mutation in SLCO1B1. Intron L1 insertion in SLCO1B3 causes skipping of exon 5 or exons 5-7 in SLCO1B3 mRNA, yields immature stop codon, and results in the generation of truncated OATP1B3 protein. OATP1B1 and 1B3 are involved in hepatic uptake of bilirubin glucuronides, bile acids, and steroidal and thyroid hormones, as well as various drugs such as statins and anticancer drugs. Therefore, their genetic abnormalities may cause acquired cholestasis. In this study we analyzed L1 insertion in SLCO1B3 and c.1738C>T mutation in SLCO1B1 in Japanese patients with drug-induced cholestasis. [Patients and Methods] A total of 44 Japanese patients with drug-induced cholestasis were enrolled after written informed consent was obtained. Inclusion criteria were (1) alkaline phosphatase (AP) greater than 2 times the upper limit of normal (ULN), (2) R, ≤ 2, when R was defined as alanine aminotransferase (ALT)/ULN divided by AP/ULN, and (3) absence of other hepatobiliary diseases. A single-tube, three-primer PCR assay was established to detect L1 insertion in SLCO1B3. The c.1738C>T mutation in SLCO1B1 was genotyped by direct sequencing. Allele frequency was compared with Japanese controls (n=554). [Results] Median (min-max) age of the cases was 65 (21-80) years and males were dominant (64%). The main causative drugs were antplatelet agents (20%), followed by cardiovascular agents (15%), anticoagulants (10%), and antidiabetic agents (10%). In SLCO1B3 polymorphism, insertion (ins)/ins, ins/wild, and wild/wild genotype was present in 23%, 20.5%, and 77.3% of the cases, respectively, while in 0.2%, 10.5%, and 89.4% of the controls, respectively. The allele frequency of L1 insertion was 12.5% of the cases, which was significantly greater than the controls [5.4%, p=0.012, odds ratio 2.5 [95% CI: 1.3-4.9]]. The c.1738C>T mutation in SLCO1B1 was not observed in both cases and controls. [Conclusions] The genotype of L1 retrotransposon insertion in SLCO1B3 was observed more frequently in Japanese patients with drug-induced cholestasis than controls. As L1 insertion potentially impairs the function of OATP1B3, the individuals with this polymorphism might be predisposed to acquired cholestasis.

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The role of repeated brush cytology in detecting dysplasia or cholangiocarcinoma in primary sclerosing cholangitis before liver transplantation

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Aim: The performance of single and repeated brush cytology in detecting dysplasia or cholangiocarcinoma (CCA) in patients with primary sclerosing cholangitis (PSC) prior to liver transplantation, and patients’ survival during follow-up was compared to the histopathology of the explanted liver. Methods: All consecutive PSC patients undergoing liver transplantation in Sweden between 1999 and 2013 were evaluated (n=255). Patients were categorized using histopathology of the explanted liver to determine the presence of CCA or dysplasia. Sensitivity, specificity, and other measures of test performance were calculated for single and repeated brush cytology, with or without fluorescence in situ hybridization (FISH). Survival after liver transplantation was analyzed using Kaplan-Meier estimate. Results: Brush cytology was done before liver transplantation in 117 of the 225 patients, of whom 65 patients were brushed more than once. The sensitivity and specificity of brush cytology for diagnosing dysplasia or CCA increased from 50% and 81% respectively in patients with one sampling, to 100% and 83% respectively in patients where repeated examinations were performed (table 1). When considering only the subgroup where FISH was also done in addition to brush cytology (n=64), the presence of aneuploidy increased the sensitivity of brush cytology in this subgroup from 83% to 95%, while the finding of only diploid cells increased specificity from 90% to 95%. Survival after liver transplantation was significantly lower in the group with pre-transplantation undiagnosed CCA in the explanted liver (p<0.001). Conclusion: In PSC patients, the utilization of repeated brush cytology or the combination with FISH results in increased sensitivity and specificity for the detection of dysplasia or CCA. Improving pre-transplantation test performance is important as the presence of undiagnosed CCA results in significantly worse survival when discovered in the explanted native liver.

Test performance for the detection of cholangiocarcinoma or dysplasia

|                | Single brush cytology | Repeated brush cytology |
|----------------|-----------------------|-------------------------|
| N              | 48                    | 65                      |
| Sensitivity    | 50%                   | 100%                    |
| Specificity    | 79%                   | 81%                     |
| ROC            | 0.645                 | 0.91                    |
| LR+            | 2.38                  | 5.33                    |
| LR-            | 0.633                 | 0                       |
| OR             | 3.75                  |                          |
| PPV            | 39%                   | 65%                     |
| NPV            | 89%                   | 100%                    |

ROC: receiver operating characteristics, LR: likelihood ratio, OR: odds ratio, PPV: positive predictive value, NPV: negative predictive value.

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Contribution of Next Generation Sequencing to the genotyping of patients with hereditary cholestasis and cholelithiasis

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Background: Next generation sequencing (NGS) allows the high-throughput sequencing of multiple genes in several subjects, concomitantly. This new technology should lower the cost and time of molecular analyses in hereditary cholestasis and cholelithiasis, which comprise autosomal dominant and recessive disorders, and the disease-causing genes of which contain numerous exons. NGS might also help to identify new genes in these disorders. Aims of the study: 1) Determine the feasibility and input of a NGS strategy for the screening of the genes implicated in the recessive disorders progressive familial intrahepatic cholestasis (i.e. ABCB4, ABCB11 and ATP8B1) and Dubin Johnson’s syndrome (i.e. ABC2C2); 2) Screen 5 candidate genes encoding transporters (ABCG5, ABCG8, SLC4A2) or bile acid receptors (NR1H4, GPBAR1). Materials and Methods: Fifty-five consecutive unrelated patients (2/3 females), referred to our national reference laboratory for intrahepatic cholestasis or cholelithiasis genotyping, were investigated. Genomic DNA was extracted from peripheral blood. A DNA capture strategy was developed, allowing the concomitant screening of 24 patients. Each DNA was converted to a sequencing library by fragmentation, end repair, and ligation to oligonucleotide adapters. Specific gene probes for the 154 coding exons of the 10 genes of interest were designed with the Nimblegen software. Individual library fragments were double captured and clonally amplified by solid surface bridge amplification (MiSeq sequencer). The dropGenTM application was used to analyze the raw data of sequencing. Results: NGS was extremely time-efficient and accurate. Each analysis of 24 patients was completed within a week. Sanger sequencing confirmed all identified variants. The mean coverage was ≥96.5% with a depth of 400X. In 37 patients, variants were detected in ABCB4 (n=14), ABCB11 (n=9), ABC2C2 (n=14) and/or ATP8B1 (n=5) genes. In 5 patients, 2 of these genes were mutated (e.g. ATP8B1/ABCB4). In 10 patients, the variant(s) of one of these genes were associated with variant(s) in one of the candidate genes, namely GPBAR1 (n=4), ABCG5 (n=5) or ABCG8 (n=1). The homozygous ABCB11 p.Val444Ala genotype, known to be associated with hormonal cholestasis, was detected in 20 patients. Conclusions: NGS provides multiple advantages over the classical methods for genotyping subjects with hereditary cholestasis or cholelithiasis. The results obtained in a small study population highlight the frequent combination of variations in genes previously known to be involved in these disorders, between themselves, and, with other genes, mainly GPBAR1 and ABCG5.

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Fenofibrate is Effective Adjunctive Therapy in the Treatment of Primary Biliary Cirrhosis: A Meta-Analysis

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Background and Aim: Fenofibrate is a novel therapy for Primary Biliary Cirrhosis (PBC). We sought to perform a systematic review and a meta-analysis of studies that assessed the efficacy of fenofibrate in the treatment of PBC patients. Methods: electronic database search was performed for relevant studies. Database searched included PubMed, Scopus, and ScienceDirect. In addition, search of abstracts presented in the main scientific meetings in the field and articles in press was performed. Random effect model was used to pool the effect size across studies for changes in levels of alkaline phosphatase, GGT, bilirubin and IgM levels before and after treatment and the overall rate of having complete response to fenofibrate therapy. Publication bias, heterogeneity testing and sensitivity analysis were also performed. Results: Six studies with 102 patients (90% female) met the inclusion criteria. All studies were case crossover where patients who had no or incomplete response to UDCA had fenofibrate added at a dose of 100-200 mg daily. Treatment duration ranged from 8-100 weeks. Treatment with fenofibrate was associated with a significant decrease in the pooled mean alkaline phosphatase (-114 IU/L, 95% CI: -152 to -76, p < 0.0001); a significant decrease in GGT level (-92 IU/L, 95% CI: -149 to -43, p = 0.0044); significant decrease in total bilirubin (-0.11 mg/dl, 95% CI: -0.18 to -0.08, p = 0.0008); and a significant decrease in IgM level (-88 mg/dl, 95% CI: -119 to -58, p < 0.0001). The pooled complete response rate was 69% (95% CI: 53-82%; p = 0.024). The odds ratio of achieving complete response while on fenofibrate was 2.43 (95% CI: 1.44-4.1, p = 0.0009). Conclusions: Fenofibrate therapy at doses of 100-200 mg daily appears to be an effective adjunctive therapy in PBC patients who had no or incomplete response to UDCA. There is a critical need for larger scale randomized trial to confirm its efficacy and define its position in the treatment paradigm of PBC.

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Fenofibrates do not improve transplant-free survival despite biochemical response in patients with primary biliary cirrhosis

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Background: Primary biliary cirrhosis (PBC) is a chronic, cholestatic liver disease that can lead to cirrhosis & liver failure. Ursodeoxycholic acid (UDCA) improves transplant-free survival, but up to 40% may not achieve adequate biochemical response. Fibrates may decrease alkaline phosphatase (ALP), but no study has examined their impact on transplant-free survival. Aim: To demonstrate whether fibrates 1) lead to biochemical response in UDCA nonresponders (drop in >40% in ALP), 2) impact bilirubin, which has been correlated with disease prognosis, 3) Impact rates of transplant-free survival & decompensated cirrhosis. Methods: 438 patients were categorized as non-responders if they had a <40% drop in ALP after one year of UDCA. A time-dependent propensity score was derived to determine the probability of patients receiving fibrates. Primary outcome measure: transplant-free survival, reaching minimal listing criteria or decompensated cirrhosis. Secondary outcome: biochemical response and change in bilirubin. Results: Of 387 eligible patients, 133/387 (34.4%) were non-responders: 49/133 (36.8%) were on a fenofibrate and UDCA (FF) and 84/133 (63.2%) on UDCA alone (UDCA). The propensity score was derived from baseline age, time from diagnosis, cirrhosis, bilirubin and ALT. Time on lipidil was 336±402 days. Those with decompensated cirrhosis had a lower mean bilirubin over time in the FF group compared to the UDCA group. In the FF group, 25/33 (75.8%) of patients had >40% drop in ALP after >100 days of treatment. Similar number of patients decompensated (19.0% UDCA; 18.4% FF, p = 1.00), died/underwent transplant (14.3% both FF & UDCA groups, p = 1.00). Conclusion: Fenofibrates lead to biochemical response, but do not have a clear impact on transplant-free survival or decompensated cirrhosis in PBC.
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Assessment of Environmental Exposures among 1000 North American Primary Sclerosing Cholangitis Patients with and without Inflammatory Bowel Disease

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Background & Aims: It has been postulated that primary sclerosing cholangitis (PSC) develops through immune mediated mechanisms triggered by complex gene-environment interactions in susceptible individuals. However, the relationships between PSC and the environment are largely unknown. While tobacco use has been reported to have a negative association with PSC, other exposures particularly dietary habits and methods of food preparation have not been well explored. Our aims were to validate or refute associations reported in previous studies and to identify novel environmental exposures among PSC patients. Methods: We performed a case-control analysis utilizing self-administered questionnaires. Cases were recruited from 8 academic medical centers across North America and controls were recruited from the Mayo Clinic during annual visits for preventive health care. Responses between cases (n=1000) and controls (n=663) were compared using multivariable logistic regression adjusted for age and gender. The model was further stratified based on inflammatory bowel disease (IBD) status (with IBD n=741; without IBD n=259). Results: A history of smoking was inversely associated with PSC only when IBD was present (OR 0.5; 95% CI 0.4-0.7) but not among PSC patients without IBD (OR 0.9; 95% CI 0.7-1.2). Moreover, women with PSC (irrespective of the presence of IBD) were less likely to have received hormone replacement therapy (HRT) (OR 0.5; 95% CI 0.4-0.7) and were more likely to have recurrent urinary tract infections (UTI's) (OR 1.6; 95% CI 1.2-2.3) when compared to controls. Furthermore, PSC patients regardless of gender or IBD status were less likely to eat fish (OR 0.4; 95% CI 0.3-0.6), vegetables (OR 0.9; 95% CI 0.8-0.9) and grilled/barbecued meat (OR 0.8; 95% CI 0.7-0.9). In contrast, PSC patients with and without IBD were more likely to consume steak/burgers that were more well-done (OR 1.3; 95% CI 1.2-1.5). Conclusions: To date, this is the largest study (which represents approximately 3% of the estimated PSC patient population in the United States) that examines environmental exposures and PSC. IBD (rather than PSC) was associated with smoking. Women with PSC were more likely to have recurrent UTI's and less likely to receive HRT. Furthermore, dietary intake and methods of food preparation differs in PSC patients when compared to controls. Estrogen, recurrent UTI's and dietary habits may be relevant to the pathogenesis of PSC and warrant further study. These findings have the potential to lay the foundations for future studies to examine the complex interactions between genes and environmental exposures in PSC.

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Efficacy Trial of All-trans Retinoic Acid (ATRA) in combination with Ursodeoxycholic Acid (UDCA) in Primary Sclerosing Cholangitis (PSC)

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Background: Effective medical therapies for PSC are needed. Moderate dose UDCA improves serum biochemistry but does not change the disease course. ATRA can activate FXR (farnesoid X receptor) and RXR (retinoid X receptor) and repress CYP7A1 and bile acid synthesis in human hepatocytes. In animal models of biliary injury ATRA improved hepatic inflammation and fibrosis when combined with UDCA (He et al. Hepatology 2011; Cai et al. J. Pharm. Exp. Ther. 2014). Aim: To determine if ATRA + UDCA improve serum parameters of cholestasis in PSC patients with alkaline phosphatase (AP) >1.5xULN despite moderate-dose UDCA for ≥6 months. Methods: Patients were enrolled at Yale and Mayo Liver Clinics. ATRA capsules were compounded and dosed at 45 mg/m2/day divided b.i.d.. Combination therapy was given for 12 weeks followed by a 12-week washout. Baseline labs were compared to the end-of-treatment and to washout. Results: Twenty-one subjects were screened and 19 enrolled. Mean age was 45±11 years, 74% Caucasian and 74% male; 63% had large duct vs. 37% with small-duct PSC, 79% had IBD and median Mayo PSC Risk Score was -0.03±0.7. Mean UDCA dose was 18±6 mg/kg/day. Fifteen subjects completed 12 weeks of therapy. Mean AP significantly declined (356±209 vs. 318±225 U/L, p=0.046); 20% achieved ≥30% reduction. Interestingly, mean alanine aminotransferase (ALT) also declined (94±55 vs. 56±32 U/L, p=0.007) along with serum bile acid levels (41±52 vs. 28±45 umol/L, p=0.04).
LDL (131±60 vs. 155±51 mg/dL, p=0.055) and triglyceride (86±31 vs. 145±45 mg/dL, p=0.003) levels increased while HDL decreased [61±21 vs. 41±11 mg/dL, p=0.01]. Mean serum levels of bile acid intermediate 7a-hydroxy-4-cholesten-3-one (C4) significantly decreased (17±19 vs. 9±11 ng/mL, p=0.04) indicating that ATRA inhibited bile acid synthesis. There was no difference in bilirubin or peripheral regulatory T cell frequency. At washout, clinical parameters and C4 levels returned to baseline (p=NS). Four subjects failed to complete 12 weeks of therapy (headache N=2, tinnitus N=1, obstructive jaundice from a known stricture N=1). The most common adverse effects were headache (63%) and tinnitus (26%). Gradual ATRA introduction over 7 days decreased headache severity and improved tolerability. Summary: Twelve-week combination therapy with ATRA + moderate dose UDCA significantly decreased serum AP, ALT, and bile acids levels. Side effects were frequent at the full ATRA dose. Conclusion: ATRA is a potent inhibitor of bile acid synthesis in humans. Further evaluation of combination therapy with a lower dosing of ATRA, UDCA and other new agents is recommended. (NCT01456468)

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294 Galectin-3 Plays a Role in Primary Biliary Cirrhosis by Mediating Inflammomassae Signaling
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Galectin-3 a lectin family member is a known mediator of stellate cell activation and liver fibrosis, and active Kupfer cells (KC) are described as a major source of galectin 3 in the liver. Macrophages play a significant role in the pathogenesis of primary biliary cirrhosis (PBC) however, the early inflammatory events are not well described. We hypothesize that the bile acid-induced galectin-3 mediates inflammation signaling in macrophages contributing to the progression of PBC. Methods: Liver tissues from PBC patients and healthy controls; and from the dNTRF II transgenic mice a model of PBC/autoimmune cholangitis and wt controls were collected for Western blot and real-time qPCR to analyze the expression of galectin-3, NLRP3, ASC and IL-1. The cleavage of caspase-1 and IL-1 in PBC patients was also examined by Western blot. For in vitro studies, primary mouse KC were isolated from wt and the galectin-3/- mice and treated with deoxycholic acid (DCA) with/without recombinant galectin-3. The cells were collected for qPCR to analyze the activation of inflammasome-related transcripts and for immunoprecipitation (IP) to detect the association of galectin-3 and NLRP3. Results: The mRNA levels of galectin-3, NLRP3, ASC and IL-1 significantly increased in the livers of the PBC patients (p<0.05, p<0.05 and p< 0.05 respectively) and in the dNTRF II mice (p<0.01, p<0.05, p<0.01 and p< 0.05) compared to the healthy controls. The protein levels of galectin-3, NLRP3 and the activation of caspase-1 and IL-1 were also elevated in the PBC patients. In wt KC, DCA significantly induced the expression of NLRP3 (p<0.05), IL-1 (p<0.01), INF (p<0.01), IL-10 (p<0.05) and IL-17A (p<0.05). However, the induction of these genes was attenuated in the galectin-3/- KC (p<0.01, p<0.001, p<0.01, 0.05 and p<0.01). Recombinant galectin-3 partially reversed the expression of the above genes in the knockout KC. Co-IP assay showed that galectin-3 associated with the NLRP3 in KC. Conclusion: Galectin-3 expression and inflammasome activation are induced in PBC patients and in dNTRF II mice. DCA induces inflammasome activation in KC resulting in the release of proinflammatory mediators, in a galectin-3-dependent manner. Galectin-3 hence could be a potential therapeutic target in PBC.

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295 An International Phase 3 Study of the FXR Agonist Obeticholic Acid in PBC Patients: Effects on Markers of Cholestasis Associated with Clinical Outcomes and Hepatoellular Damage
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Obeticholic acid (OCA, 6-ethyl chenodeoxycholic acid) is a highly potent, selective FXR agonist. In Ph2 PBC studies, 10-50mg OCA (±UDCA) produced significant biochemical improvement in cholestasis and inflammatory markers. The Global PBC Study Group (GPBCSG) confirms patients with alkaline phosphatase (ALP) >1.67x ULN or bilirubin >ULN have a greatly increased risk of liver transplant or death [HR (95% CI): 2.83 (2.4-3.4); p =1x10^-6). This was an international, double-blind, placebo-controlled (PBO) trial. PBC patients ≥ULN or bilirubin <2x ULN were randomized to PBO, OCA 5 or 10mg for 12mo. Patients randomized to 5mg were titrated to 10mg after 6mo, if necessary; pre-study UDCA continued. The primary endpoint was attaining the GPBCSG ALP/Bilirubin goal of OCA treated patients achieved the ALP/Bili response goal prior to PBO, a highly statistically significantly greater proportion of OCA treated patients achieved the ALP/Bili response goal (see table). After 6mo, ALP significantly improved (p<0.0001) with OCA dose: PBO, -6.8% ±3.5), 5mg, -27.4% ±3.4), 10mg, -36.5% ±3.5). Pruritus, generally mild to moderate, was the most common and dose related adverse event (AE). The incidence of AEs other than pruritus was no worse with
OCA (PBO, 90%, 5/10mg OCA, 89%, 10mg OCA, 86%). An 82-yr old patient with pre-existing congestive heart failure taking OCA died due to worsening of the condition. Overall, serious adverse events (SAEs) occurred in 22 (10%) of patients and, although there were more SAEs in the OCA groups, none were considered drug-related and there were no apparent patterns. Modest mean reductions in HDL (16%/5/10mg OCA, 26%/10mg OCA) were observed with OCA. OCA produced highly statistically, clinically meaningful improvements in biochemical criteria that are strongly correlated with clinical benefit. Pruritus was the principal AE, but had a lower incidence in the titration group compared to 10 mg. Starting patients on 5mg OCA and titration to 10mg based on the clinical response appears to be an appropriate dosing strategy.

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**296 Noninvasive testing are poor surrogate markers for fibrosis staging and liver-related outcomes in patients with primary biliary cirrhosis who do not respond to ursodeoxycholic acid**

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**Background:** Primary biliary cirrhosis (PBC) is a chronic, cholestatic liver disease that can lead to cirrhosis and liver failure. Few studies have examined the utility of noninvasive tests (NITs) of fibrosis in PBC. Aim: To determine the accuracy of noninvasive tests (FIB-4, AST/ALT, APRI, ultrasound) in predicting advanced fibrosis as compared with liver biopsy. Methods: 387 charts were assessed and nonresponders (patients with <40% decline in alkaline phosphatase (ALP) after 1 year of treatment with ursodeoxycholic acid) with liver biopsy, FIB-4, AST/ALT and APRI were included. The annual mean value for noninvasive markers of fibrosis were calculated using values taken within a year of their liver biopsy. Using ordinal regression and ROC curves, we determined the predictive accuracy of NITs as compared to liver biopsy (primary outcome). Secondary outcome measure: Correlation with end of follow-up outcomes. Results: 72 patients met inclusion criteria. Patients were dichotomized into histologically defined early (stage 0-2; EF) and late fibrosis (stage 3-4; LF), with 51 and 21 patients in each respective group. Median age at diagnosis was 47.0 and 43.1 years (EF and LF groups respectively). Patients were followed for a median 11.7±6.3 (EF) and 11.9±7.2 years (LF). Approximately 19% of patients compensated, died or underwent transplant/met minimal listing criteria in both groups. AST/ALT was not a significant predictor of advanced fibrosis on biopsy (p=0.154), in contrast to APRI (p=0.019) and FIB-4 (0.026). The AUROCs for AST/ALT, APRI, FIB-4 were 0.599, 0.602 and 0.636 respectively. Ultrasound performed poorly, with an AUROC of 0.654 even in the presence of portal hypertension. No test, including liver biopsy, was predictive of survival despite up to 24 years of follow-up. Conclusion: Survival in patients with PBC, even in cirrhotic patients who do not respond to UDCA, is in excess of 10-15 years, which emphasizes the challenge in using clinical endpoints as outcome measures in clinical trials. Non-invasive testing does not accurately predict the presence of fibrosis in patients with PBC. Ultrasound performs poorly and should not be used to diagnose cirrhosis in this population.

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Applicability and Prognostic Value of Histologic Scoring Systems in Primary Sclerosing Cholangitis

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Introduction

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease, affecting intra- and extrahepatic bile ducts. Common hepatic histologic changes include inflammation, accumulation of copper binding protein (CBP), ductopenia and concentric periductal fibrosis. At present there is no PSC-specific histologic scoring system to evaluate both disease activity and progression. The aim of this study was to assess if three scoring systems designed primarily to assess disease severity in chronic hepatitis (Ishak 1995) or PBC (Ludwig 1978, Nakanuma 2010) could also be used for grading and/or staging PSC. Methods

For this cohort study, PSC patients from a Dutch population-based cohort, who underwent diagnostic liver biopsy were included. Two expert liver pathologists evaluated biopsy slides in tandem. Grading was scored using the Nakanuma system (cholangitis activity, hepatitis activity) and the Ishak system. Staging was scored using the Nakanuma system (fibrosis, bile duct loss, CBP deposition) and the Ludwig system (cirrhosis, biliary inflammation, accumulation of copper binding protein (CBP), ductopenia and concentric periductal fibrosis). Association of grading and staging with transplant-free survival, as well as time to liver transplantation (LTx) alone was estimated using Kaplan Meier survival curve and log rank test. Results

Sixty-four patients were included, with a median follow up of 12 months (IQR 71-179). Mean age at diagnosis was 38 years (±14), 63% were male. Forty-four patients (69%) had large duct PSC and 43 (67%) had concomitant inflammatory bowel disease (IBD). A total of 9 patients reached an endpoint (7 LTx, 2 death from CCA) in a median time of 103 months (IQR 34-160). During grading and staging of biopsies, consensus was reached in 100% of cases. Histologic grading according to Ishak was highly significantly associated with time to Ltx (p=0.007). Histologic staging of fibrosis and CBP deposition (dichotomized), according to Nakanuma was significantly associated with transplant-free survival (p=0.006 and p=0.01 respectively). Ishak and Ludwig staging scores also showed a statistically significant association with transplant-free survival (p<0.001 and p<0.001 respectively). Conclusion

The Nakanuma, Ishak and Ludwig scoring systems are applicable to PSC liver biopsies. A significant association was shown between Ishak grade and time to Ltx. Staging of PSC using all three systems is highly associated with transplant-free survival. Our observations suggest that these staging systems may be useful in the evaluation of disease severity and as response parameters to therapeutic interventions in PSC patients.

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Relation of gallbladder enlargement with bile acid homeostasis and colorectal malignancy in primary sclerosing cholangitis

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Background and aim: Gallbladder enlargement is frequent in primary sclerosing cholangitis (PSC). In mice, bile acid homeostasis can be modified by a gallbladder shunt. The aim of this study was to assess the potential cause and influence of gallbladder enlargement on bile acid homeostasis and disease course in PSC.

Patients and methods: The study population comprised 77 PSC patients who underwent a three-dimensional magnetic resonance cholangiography (3D-MRC) and a mass spectrometry analysis of serum bile acids within less than a month. Patients were followed for 9.5 years. Gallbladder volume was calculated by the analysis of 3D-MRC images and the median value [69 mL] was taken as a cutoff to separate patients into two groups. Results: Gallbladder was enlarged in 35 patients [89 [86-106] mL] and within normal range in 42 [41 [35-47] mL]. Patients with enlarged gallbladders did not significantly differ from others regarding gender [65% vs. 66% males], median age [43 vs. 42 years], time from diagnosis [5 vs. 5.5 years], body mass index [21.6 vs. 23.7], associated inflammatory bowel disease [71% vs. 50%], UDCA treatment [89% vs. 90%], other MRI features including cystic abnormalities [8.5% vs. 12%], clinical or histological parameters of liver disease [Mayo risk score of -0.18 [0.45-0.27] vs. -0.005 [0.63-0.56]). Notably, malignancy was less frequent in the group with enlarged gallbladder, occurring in 2 [5.7%] vs. 11 [26.2%] patients with normal gallbladder size (P=0.029). Colorectal cancer in particular was 6.7-fold less frequent, occurring in 1 [2.8%] vs. 8 [19%] patients (P=0.037, OR=6.7 [0.9-354]). In patients with enlarged gallbladder, the serum concentrations of secondary bile acids were lower than in other patients [1.6 [1.3-1.9] vs. 2.5 [2.3-3.9] μmol/L, P=0.0004). This was true for deoxycholic acid [0.7 [0.5-1.1] vs. 2.2 [1.6-3.6] μmol/L, P=0.0001), a secondary bile acid known to promote colon carcinogenesis. Patients in this group also had higher concentrations of primary bile acids [10.5 [6.6-16.7] vs. 4.3 [3.5-5.3] μmol/L, P=0.0001] and of UDCA [44.0 [29.4-52] vs. 27.2 [14.6-31.1] μmol/L, P=0.001]. Furthermore, they had higher serum concentrations of the gallbladder-relaxing hormone FGF19 [211.6 [168.6-234.6] vs. 88.6 [72.7-121.6] pg/mL, P=0.0001), which concentration was correlated with gallbladder volume [R2=0.46, P=0.001]. Conclusion: Gallbladder is enlarged in approximately half of PSC patients, which can be caused by increased FGF19 levels, and which is associated with a lack of secondary bile acids, enhanced UDCA enrichment and a lower prevalence of colorectal cancer, consistent with protective properties of gallbladder enlargement in PSC.

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A possible involvement of endoplasmic reticulum stress in the process of biliary epithelial autophagy and senescence in primary biliary cirrhosis

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Background/Aims: Accumulating data suggest that deregulated autophagy followed by cellular senescence in biliary epithelial cells (BECs) may be closely related to the abnormal expression of mitochondrial antigens and following autoimmune pathogenesis in primary biliary cirrhosis (PBC). Given endoplasmic reticulum (ER) stress affect a process of autophagy, we hypothesized that ER stress may be involved in the deregulated autophagy and cellular senescence in biliary epithelial lesions in PBC.

Methods: We examined the expression of ER stress markers [GRP78, CHOP, XBP-1, spliced form and protein disulfide isomerases; PDI] at mRNA and protein levels in cultured BECs treated with glycochenodeoxycholic acid (GCDC, 200 μM), palmitic acid (PA, 200-400μM; control, oleic acid) and ER stress inducer, tunicamycin (TM, 0.5μg/ml). The effect of pretreatment with tauroursodeoxycholic acid (TUDCA, 1 mM) on the induction of ER stress was also examined. The degree of autophagy and cellular senescence in cultured BECs treated with GCDC, PA, TM was assessed using the immunoblot of microtubule-associated proteins-light chain 3 (LC3), Immunocytochemistry for p62/suquestosome-1 (p62) and a detection of senescence-associated -galactosidase (SA-)gal activity.

The expression of ER stress markers was significantly increased in cultured BECs treated with GCDC, PA and TM (p<0.05). Pretreatment with TUDCA significantly suppressed ER stress in BECs treated with GCDC, PA and TM (p<0.05). Autophagy, deregulated autophagy with p62 accumulation and cellular senescence were induced in cultured BECs treated with GCDC, PA and TM. Pretreatment with TUDCA further increased the degree of autophagy in BECs treated with GCDC, PA and TM. Pretreatment with TUDCA suppressed the stress-induced cellular senescence in cultured BECs (p<0.05). An intense granular and vesicular expression of ER stress markers, PDI and GRP78, was seen in damaged small bile ducts (SBDs) in PBC. The expression of PDI and GRP78 was significantly more extensive in SBDs in PBC, compared with control livers (p<0.05). The expression of ER stress markers was correlated with the expression of LC3 and p16INK4a and p21WAF1/Cip1 in PBC. In conclusion, ER stress may play a role in the pathogenesis of deregulated autophagy and cellular senescence in biliary epithelial lesions in PBC.

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301 Younger patients presenting with Primary Biliary Cirrhosis are more likely to have cognitive impairment

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Introduction: Data from the UK-PBC cohort have shown that patients presenting with Primary Biliary Cirrhosis (PBC) at a younger age have a greater symptom burden, particularly fatigue and autonomic dysfunction. Previous studies have demonstrated that cognitive dysfunction is prevalent in PBC. Aim: To evaluate the prevalence of cognitive impairment in the UK-PBC patient cohort and identify relevant associations. Methods: The UK-PBC dataset was analysed. This observational study used the cognitive domain of the PBC-40, the Orthostatic Grading Scale (OGS) and the Epworth Sleepiness Scale (ESS). Results: Data on 2187 patients were analysed. 27% of PBC patients had clinically significant cognitive impairment. Patients without evidence of advanced liver disease (normal bilirubin and albumin) had a higher prevalence of clinically significant cognitive impairment (37%) than the group as a whole. Paradoxically, given the positive correlation between age and cognitive dysfunction in the normal ageing population, cognitive dysfunction was significantly associated with both a younger age at diagnosis (r = -0.14, p < 0.0001) and a younger age at entry into the study (r = 0.11 p < 0.0001). This is compared with a positive correlation of r = 0.103 seen in an age and sex-matched community control population. Patients under 50 years old at presentation had significantly more cognitive dysfunction than those >60 (35% vs 19%, CS = 10.5, p < 0.0001). Cognitive dysfunction was not associated with duration of disease or response to ursodeoxycholic acid. Autonomic symptoms were strongly associated with cognitive symptoms in both <50 and >60 patients (<50: mean OGS 5.5 ± 3.7 with cog symptoms v 2.3 ± 2.6, p < 0.0001). ESS scores were significantly higher in the <50 patients with significant cognitive symptoms than >60 (p = 0.001). Cognitive impairment was associated with significantly increased social dysfunction in younger patients compared to older (62% vs 42%, CS = 10.5, p = 0.001). This is important as we know social dysfunction is closely linked to perceived quality of life in PBC. Conclusions: Cognitive dysfunction is frequent in PBC and, contrary to expectation, is significantly commoner in patients presenting at a younger age. This argues against cognitive dysfunction in PBC simply being a manifestation of advancing age or hepatic encephalopathy. The cognitive impairment seen in younger patients may in part be due to additional sleep disturbance in this age group, with autonomic dysfunction contributing in both age groups. Cognitive dysfunction is under-recognised and unappreciated and warrants further research.

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302 Ongoing Activation of Autoantigen-Specific B cells in Primary Biliary Cirrhosis

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Background. Antimitochondrial antibodies have served as a fingerprint to identify mechanisms that lead to loss of tolerance in primary biliary cirrhosis (PBC). AMAa recognize not only the E2 component of pyruvate dehydrogenase (PDC-E2), but also chemical xenobiotics with structural similarity to the inner lipoyl domain of PDC-E2. It is unclear whether such autoantibodies result from de novo activated autoantigen-specific B cells, or from B cells primed when self tolerance was compromised. We examined B cell and plasmablasts phenotype, frequency, isotype and pattern of autoantibody reactivity in PBC and controls by comparing binding activities to PDC-E2 and to two representative etiologically associated xenobiotics, 2-octynoic acid (2OA) and 6, 8-bis (acetylthio) octanoic acid (SAC). Methods. Firstly, using a total of 58 subjects, including 27 with PBC, 13 with PSC and 18 healthy controls, we monitored the frequencies of B cell subsets and focused on the frequency of PDC-E2 specific responses of plasmablasts by ELISPOT. Additionally, we analyzed the plasmablast-derived antibodies compared to plasma antibodies to quantitate reactivity against PDC-E2, 2OA and SAC. Finally, we characterized the phenotype of PDC-E2-specific plasmablasts. Results. Importantly, the frequencies of total B cells, naive B cells, class-unswitched memory B cells, and class-switched memory B cells are similar between PBC and controls. Strikingly, however, 10% of the total IgG and IgA plasmablast and 23% of the IgM plasmablast population were uniquely reactive with PDC-E2 in PBC (p < 0.01). Plasmablast reactivity to the control antigen, tetanus toxoid, was similar in all groups, indicating that PDC-E2-specific antibody secreting cells represent newly activated plasmablasts, rather than re-activation of the pool of PDC-E2-specific memory B cells. Plasma antibodies from PBC, but not controls, reacted to PDC-E2, 2-OA and SAC. In contrast, the plasmablast-derived polyclonal antibodies from PBC reacted with PDC-E2, but did have detectable reactivity against 2OA and SAC. Interestingly, PDC-E2-specific plasmablasts expressed the homing receptors, CXCR7 and CCR10, suggesting a mechanism for the migration of PDC-E2-specific plasmablasts to the epithelial ligands, CXCL12 and CCL28. Conclusions. The dramatic elevated frequency of circulating plasmablasts specific for PDC-E2, but not reactive to xenobiotics, is consistent with an ongoing intense activation of autoantigen-specific B cells by cognate antigen. Finally, this chronic and intense response suggests that immunotherapeutic approaches in PBC must focus on the original forbidden sin, the loss of tolerance to PDC-E2.

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Risk stratification in primary biliary cirrhosis using the UK-PBC Research Cohort
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BACKGROUND: UDCA response predicts outcome in primary biliary cirrhosis (PBC). However, existing predictive models dichotomise UDCA response and fail to recognise a continuum of risk. Also, risk stratification based on UDCA response does not take the stage of the liver disease into account. We analysed data from the UK-PBC Cohort to determine whether variables reflecting disease stage might improve current prognostic models; and to compare models treating UDCA response as a continuous versus categorical variable.

METHODS: We undertook time-to-event analysis using the Cox proportional hazard regression model. The entry point was the date of presentation and the endpoint was the date of failure (liver transplant for, or death from, PBC-related liver failure). The Akaike information criterion (AIC) was used as model-selection criterion; the model with the lower AIC was considered the model that better fits the data.

RESULTS: Data were available for 2274 PBC patients treated with UDCA for at least one year. On multivariate analysis, the liver biochemistry after one year of UDCA most strongly predicted failure, i.e. bilirubin (P = 1.31 x 10^-19), transaminases (P = 1.92 x 10^-12) and alkaline phosphatase (P = 0.003). However, variables reflecting disease stage had effects independent of UDCA response, i.e. baseline bilirubin (P = 0.0002), creatinine (P = 0.0102), albumin (P = 0.0001), platelet count (P = 0.0006) and splenomegaly (P = 0.0005) (Table 1). Modelling UDCA response with continuous variables (AIC = 1228) fitted the survival data better than modelling UDCA response as a dichotomous variable, e.g. Paris I (AIC = 2614), Paris II (AIC = 2626) and Toronto criteria (AIC = 2685). CONCLUSION: Treatment of PBC should undoubtedly be guided by the biochemical response. However, risk assessment might be improved by taking the stage of the liver disease into account and by recognising that the biochemical response is a continuum. Further work will be to develop continuous risk models so that treatment may be directed towards achieving maximal reduction in risk.

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NGM282 is a Potent Modulator of Bile Acid Synthesis in Humans via Suppression of CYP7A1 Activity
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Background and Aims: NGM282 is an engineered recombinant protein variant of the ileal hormone human fibroblast growth factor 19 (FGF19) which down-regulates the classical pathway of bile acid (BA) synthesis by specifically suppressing hepatic CYP7A1. NGM282 decreases serum concentrations of 7a-hydroxy-4-cholesten-3-one (C4), a surrogate biomarker for CYP7A1-mediated BA synthesis, in both mice and monkeys. The direct activity of NGM282 on BA synthesis in humans was evaluated by measuring serum C4 concentrations in a Phase 1 clinical study.

METHODS: NGM282 was dosed daily in the morning (AM) over 6 consecutive days at 0.1, 0.3, 1 and 3 mg vs placebo. Serum C4 and total BAs were measured pre-meal (fasted) and 4.5 hours post-meal (fed) at Screening (Day -1) and on Day 7. C4 and total BAs levels were collected for pharmacokinetic (PK) calculations and correlation to pharmacodynamic markers. Quantification of C4 was performed using liquid chromatography electrospray ionization tandem mass spectrometry with stable-isotope dilution analysis.

RESULTS: NGM282 significantly decreased serum C4 levels in a dose-dependent manner from Day -1 to Day 6 in both fasted and fed states at the 0.3, 1 and 3 mg dose (Figure 1) and consistent with the magnitude of change observed in animal models. Median % change from Day -1 ranged from a 28-95% decrease fasted and a 64-95% fed vs a 16-51% increase with placebo. Dose-dependent decreases in C4 were consistent with the observed dose proportional PK of NGM282. Maximal biologic activity was seen in all subjects dosed with 3 mg where as “no effect” dose was at 0.1 mg.

CONCLUSIONS: Administration of NGM282 resulted in a rapid and potent suppression of C4 in healthy human
subjects, reflective of decreased BA synthesis via the classical pathway. These data support the potential therapeutic activity of NGM282 in BA-related cholestatic disorders. Exploratory studies are currently underway in patients with primary biliary cirrhosis.

Figure 1. Median Serum C4 Concentrations

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Obeticholic Acid in PBC Patients: The Utility of Titration Based on Therapeutic Response and Tolerability

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The Phase 3 POISE trial evaluated the efficacy and tolerability of obeticholic acid (OCA), a derivative of chenodeoxycholic acid and potent farnesoid-X receptor agonist, in patients with PBC. The primary endpoint was defined as alkaline phosphatase ≤1.67xULN and a ≥15% ALP reduction and a bilirubin ≤ULN which was achieved by a significantly higher proportion of patients treated with OCA compared to placebo (p<0.0001). The aim of this analysis was to evaluate if titration of OCA would improve tolerability while remaining efficacious. This was an international, double-blind, placebo-controlled (PBO) trial. PBC patients ≤UDCA (if taking UDCA, on a stable dose) with ALP≥1.67x ULN or bilirubin ≥2x ULN were randomized to PBO, OCA 5 or 10mg for 12mo. Patients randomized to 5mg were titrated to 10mg after 6mo if 5 mg was well tolerated and the primary endpoint had not been met. This analysis focuses on the efficacy and tolerability of OCA in those subjects randomized to 5 mg OCA who subsequently were or were not titrated to 10 mg. All groups were well-matched. Mean age: 55.8yrs, female: 91%, Caucasian: 94%, 7% were not taking UDCA. Overall, 91% of patients completed the study. The titration arm showed comparable efficacy to the 10 mg group with a lower overall incidence of pruritus (table). Of the 69 5 mg OCA subjects who completed 6 mo, 33 titrated to 10 mg resulting in 13 additional responders by 12 mo. 4 subjects who were eligible to titrate did not due to pruritus. One subject discontinued due to pruritus after up-titration to 10 mg. OCA given to PBC patients with an inadequate response to or unable to tolerate UDCA produced highly statistically, clinically meaningful improvements in liver biochemistries which have been shown to be strongly correlated with clinical benefit. Titration of OCA based on therapeutic response and tolerability mitigated pruritus while maintaining efficacy.

| % Responders | Baseline and Change in ALP (UL) | Pruritus (%) |
|--------------|--------------------------------|-------------|
| 6 months     | 12 months                      | Pruritus (%)|
| Placebo (n=73) | 7% | 10% | 327(15) | 18(8) | 11(11) | 38% |
| 10 mg OCA (n=73) | 51% | 47% | 316(12) | 111(10) | 118(9) | 70% |
| Titration (n=70) | 34% | 46% | 326(14) | 87(10) | 104(11) | 56% |

Titration Subgroups

| Remained at 5mg (n=36) | 67% | 53% | 307(20) | 8(11) | 8(12) | 58% |
| Titrated to 10 mg (n=33) | 0% | 39% | 34(19) | 92(16) | 126(17) | 55% |

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306 Clinical Course And Outcome Of Patients With Sclerosing Cholangitis In Critically Ill Patients (Sc-Cip): High Mortality Without Liver Transplantation

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Background: Sclerosing cholangitis in critically ill patients (SC-CIP) is a progressive biliary disease developing in a subgroup of patients during intensive care treatment. It is characterized by biliary casts/obliterration, formation of strictures and destruction of intrahepatic bile ducts consecutively leading to liver cirrhosis and liver failure. Aim of the study was to characterize clinical course, outcome and prognostic features of patients with SC-CIP. Patients and Methods: 49 patients (34 male, age: 46.0±14.2, [mean±SD, years]) with SC-CIP, diagnosed by endoscopic retrograde cholangiography (ERC) were retrospectively analyzed. No patient had evidence of preexisting hepatobiliary disease or inflammatory bowel disease. Histological evaluation of liver biopsies, ICU and endoscopic treatment as well as outcome were evaluated. Results: Respiratory failure (N=11), severe polytrauma (N=9), sepsis (N=7), lung transplantation (N=5), surgery (N=5), cardiopulmonary resuscitation (N=4) and burn injuries (N=3), were the most common reasons for hospitalization. Mean duration of hospitalization at the ICU was 73.9±44 days. All patients required prolonged mechanical ventilation (mean duration: 45±28 days), with the need of extracorporeal membrane oxygenation (ECMO) in 11 patients. Laboratory findings at the time of ERC were: bilirubin: 14.9±4.18 [mg/dl, median[range]], GGT: 29±36.08 [xULN, median[range]], AP: 10.8±28.0 [xULN, median[range]]. Sphincterotomy, extraction of casts/sludge and dilations of dominant strictures were performed during ERCP. During follow up 26 patients died and 4 patients were transplanted. Number of organ failure and organ replacement therapy were independent risk factors for mortality. Conclusion: Critical reduction of hepatic oxygen delivery may lead to initial bile duct injury in ICU patients. Vasopressor treatment and sedoanalgesia, hepatic ischemia, as well as translocation of endotoxins and bacteria from the gut may further perpetuate progressing SC-CIP, which carries a high mortality as a high proportion of these patients rapidly develop liver cirrhosis and liver failure.

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307 Successful Immunotherapy of Autoimmune Cholangitis by Adoptive Transfer of Foxp3+ Regulatory T Cells

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Background: dnTGFβRII mice develop high titer AMAs and histologic features characteristic of autoimmune cholangitis, with striking similarities to human PBC. There is increasing interest in the potential use of regulatory T cells (Tregs) as immunotherapy to treat diseases characterized by loss of tolerance. We have taken advantage of the dnTGFβRII model and, in particular, an ability to induce autoimmune cholangitis in Rag1−/− recipients by adoptive transfer of dysregulated CD8+ T cells from dnTGFβRII mice. Such adoptively transferred Rag1−/− recipients develop severe portal inflammation with both histologic and cytokine evidence of intense inflammation. Methods: Rag1−/− mice, at four weeks of age, received either CD8+ T cells from dnTGFβRII mice with co-transfer of either Foxp3+ Tregs derived from wild-type and otherwise healthy C57BL/6 mice, or dnTGFβRII mice. Recipient mice were monitored for histology including portal inflammation and intra-lobular biliary cell damage, phenotypic changes in recipient lymphoid populations and local and systemic cytokine production. Results: Adoptive transfer of CD8+ T cells into Rag1−/− recipients led to the characteristic autoimmune cholangitis at approximately 8 weeks following transfer. Importantly, co-transfer of CD8+ T cells from dnTGFβRII mice with Foxp3+ Treg cells from dnTGFβRII mice did not alter this adoptive transfer of immunopathology. However, and of striking importance, co-transfer of CD8+ T cells from dnTGFβRII mice with wild-type Foxp3+ T cells from C57BL/6 mice, significantly reduced the immunopathology, including portal inflammation, bile duct damage, and dramatic down-regulation of the secondary inflammatory response. In addition, to focus on the mechanisms of action of the ability of C57BL/6 Tregs to reduce autoimmune cholangitis, we noted significant differential expression of GARP, CD73, CD101, and CD103 and a functionally significant increase in IL-10 in Tregs from C57BL/6 compared to dnTGFβRII mice. Conclusion: These data highlight the therapeutic potential of Treg cells in reducing the excessive autoreactive T cell responses in this murine model of primary biliary cirrhosis and reflects a novel venue for treatment of patients who have undergone a breach of tolerance.

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Protein Profiles of Apoptotic Bodies from Biliary Epithelial Cells
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Background. There is increasing data suggesting a role for the apoptotic blebs of biliary epithelial cells (BECs) as a causative mechanism that leads to selective biliary destruction and an intense pro-inflammatory micro-environment. Methods. We have isolated and analyzed apoptotic bodies from normal human BECs, renal tubular epithelial cells (HRPTEpiC), bronchial epithelial cells (BrEPC) and BECs from primary biliary cirrhosis (PBC) and controls by comparative shotgun proteomics using columns coupled to a LTQ ion trap mass spectrometer nanospray source; samples were isolated and run independently. Tandem mass spectra were evaluated using the Uniprot database and pathway analysis using The Pathway Interaction NCI Database (http://pid.nci.nih.gov) as well as the STRING (Search Tool for Retrieval of Interacting Genes) software (http://string-db.org/). Results. A total of 40,843 distinct peptides and 6,160 protein groups were identified within apoptotic bodies from HiBEC, BrEPC, and HRPTEpIC. Similar numbers were identified in BECs from PBC and controls. Interestingly, 11 proteins were found to be specific for apoptotic bodies of HiBEC. Eight proteins were unique to apoptotic bodies from BrEPC and HRPTEpIC, and absent from HiBEC. Further, comparison of the global proteome of apoptotic bodies to that of intact cells from HiBEC, HRPTEpIC, and BrEPC identified a total of 3,152 protein groups within HiBECs, HRPTEpICs, and BrEPCs. Of the 11 proteins uniquely found in the apoptotic bodies of HiBEC cells, 4 of the 11 (ANXA6, LR1P, PAPS2, and SERPH) were found to be present in all three intact cell lines. One protein, HSFP6, was found only in intact HiBEC, but not intact HRPTEpIC or BrEPC. However, the other 6 proteins uniquely found in blebs of HiBEC (A6N9NB0, B4DN38, GFCO, Q6ZR44, RAB11A AND VGF3R3), were not found in intact cells. Finally, of the 3,152 protein groups, only 3 groups were found in intact HiBEC cells, but not in HiBEC apoptotic bodies (ANXAZ3, PYGB, and ITPR5). Six proteins were found to be specifically located in apoptotic bodies from PBC compared to apoptotic bodies from controls and only 2 proteins were unique to apoptotic bodies from controls that are absent in those from PBC. Analysis of the cellular pathways in HiBEC and found in apoptotic bodies identified essential inflammation pathways, including the Notch signaling pathway, IL8 and CXCR2-mediated signaling, integrin signaling, and proteins that regulate cell growth and division. Conclusion: The signature proteins identified by this unique technology implicate specific pathways that may shed light on potential therapeutic intervention.

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Efficacy of Obeticholic Acid In Primary Biliary Cirrhosis as Assessed by Response Criteria Associated With Clinical Outcome: A Poise Analysis
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Aim: Obeticholic acid (OCA, 6-ethyl chenodeoxycholic acid) is a highly potent, selective FXR agonist. Efficacy and safety of OCA was evaluated in an international double-blind placebo (PBO) controlled trial (POISE). The Global PBC Study Group (GPBCSG) confirms patients with alkaline phosphatase (ALP) >1.67x ULN or bilirubin >ULN have a greatly increased risk of liver transplant or death [HR (95% CI): 2.83 [2.4-3.4]; p =1x10-34]. Additional prognostic criteria are associated with clinical outcomes in PBC patients. This analysis evaluated the efficacy of OCA per these criteria. Methods: POISE was conducted in PBC patients ≤UDCA (if taking UDCA, on a stable, continuing dose) with ALP>1.67xULN or bilirubin <2xULN; subjects were randomized to PBO, OCA 5 or 10 mg for 12 mo. Patients randomized to 5 mg were titrated to 10mg after 6mo, based on response and tolerability. The primary endpoint was attainment of GPBCSG ALP/Bilirubin goal and ALP reduction ≥15%. Disease severity criteria of Paris I, Paris II, and Rotterdam were also assessed. Results: All groups were well-matched. Mean age: 55.8yrs, female: 91%, Caucasian: 94%. The median UDCA dose was 15.4 mg/kg; 7% were UDCA-intolerant. Overall, 91% of patients completed the study. The primary endpoint was achieved: significantly greater proportion of OCA treated patients achieved the primary endpoint. Results based on additional criteria are presented in the table. Pruritus, generally mild to moderate, was the most common and dose related AE; few OCA patients withdrew due to pruritus (<6%). The incidence of AEs other than pruritus was no worse with OCA (PBO, 90%, 5/10 mg OCA, 89%, 10 mg OCA, 86%). Overall, serious adverse events (SAEs) occurred in 22 (10%) of the patients and, although there were more SAEs in the OCA treatment groups, none were considered drug-related and there were no apparent patterns in the SAEs. Conclusions: OCA given to PBC patients with an inadequate response to or unable to tolerate UDCA produced highly statistically, clinically meaningful improvements according to several disease severity criteria, which have been shown to be strongly correlated with clinical benefit. No significant changes were seen according to the Rotterdam criteria, likely due to the high percentage of normal patients.
Table 1: POISE Endpoints

| Endpoint          | Parts I | Parts II | Rotterdam |
|-------------------|---------|----------|-----------|
|                   | Base    | Base     | Normal    | Moderate | Severe   |
| 12 Mo             | 10      | 1         | 4         | 77       | 60       | 18       | 22       | 5        | 14       |
| OCA=BR<5-10mg=BR>10mg (p<0.001) | 46*     | 52        | 0         | 27**     | 83       | 70       | 17       | 19       | 0        | 3        |
| OCA=BR>10mg (p=0.0001) | 47*     | 52        | 0         | 26*      | 82       | 63       | 15       | 21       | 3        | 1        |

POISE endpoint analysis: Patients with ALP <1.67xULN, 2-15% ALP reduction and normal bilirubin. BR=Parts I criteria: ALP >= 2x ULN and AST < 2.4x ULN and total bilirubin < ULN; Parts II criteria: ALP >= 2x ULN and AST < 2x ULN and total bilirubin < ULN. Rotterdam criteria: Normal: Normal bilirubin and albumin; Moderate: Abnormal bilirubin or albumin and Severe: Both bilirubin and albumin were abnormal.

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310 FXR Agonist Obeticholic Acid: Pruritus, A Common Side Effect Ameliorated by Dose Titration

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Obeticholic acid (OCA), a potent, selective FXR agonist in development for PBC produced significant improvement in cholestasis markers in Phase2 PBC trials at OCA 10 – 50 mg (≤UDCA). Pruritus, a hallmark PBC symptom of unknown etiology, was the most frequently occurring dose-related AE resulting in early discontinuation in up to 24% at 50 mg. In a Phase3 PBC trial (POISE), lower doses also resulted in clinically and highly statistically significant liver biochemistry improvement (<0.0001 vs. Placebo). POISE treatment emergent (TE) pruritus is characterized here. In a 1 yr, international, double-blind, placebo-controlled trial, 217 PBC patients (ALP≥1.67xULN/ TBili > ULN) were randomized to Placebo (PBO), OCA 5 mg or 10 mg. Patients randomized to 5 mg were titrated to 10 mg after 6 mo (TITR) based on biochemistry/tolerability; pre-trial UDCA continued. Baseline pruritus was recorded using a severity scale (Mild/Moderate/Severe). TE Pruritus was recorded using AE data and a visual analogue scale (VAS). Protocol-allowed pruritus interventions included drug interruption, dose interval change and medications. Pruritus, mostly mild to moderate, was the most common dose-related AE (PBO, 38%, TITR, 56%, 10mg, 68%). Only 1 (1%) of patients in the TITR group discontinued due to pruritus (after starting 10mg), vs 10% in the 10 mg group. Incidence and severity of TE pruritus improved during 2nd half of the trial (Table). Overall, <6% withdrew due to pruritus. Although on-Study VAS scores initially were higher in OCA patients vs PBO (p = 0.0005 at w2 and 0.0314 at 6 mo in 10 mg OCA), the difference was not statistically significant in the TITR arm and negligible for both arms at 12 mo vs PBO. Starting treatment at 5mg and increasing to 10mg, if appropriate, ameliorates OCA related pruritus while maintaining efficacy.
Background and Aims: Until now, prevalence of hepatocellular carcinoma (HCC) in patients with primary biliary cirrhosis (PBC) is established in UK, USA and Japan, and suggested that many factors were associated with the development of hepatocellular carcinoma (HCC) in patients with primary biliary cirrhosis (PBC). However, limited data is available regarding the clinical characteristics of PBC-associated HCC patients in China. This study was designed to investigate the clinical features of PBC-associated HCC patients in China, and further analyze its relative risk factors. Methods: Clinical data of 1255 PBC patients including 52 HCC patients in our hospital from January 2002 to December 2013 were collected and analyzed.

Moreover, a case-control study, including 20 PBC-associated HCC patients and 77 PBC patients without HCC, was conducted to analyze the risk factors for HCC development in PBC patients. Results: In our study, the total HCC incidence in Chinese PBC patients was 4.13% (52/1255), and further study showed that there was higher HCC incidence in male patients than that in female patients (9.52% vs 3.31%, P < 0.05). Higher proportion of blood transfusion, alcohol intake and smoking occurred in male PBC-associated HCC patients, and they suffered from greater degree of liver injury as indicated by higher levels of ALT, AST, AIP, and GGT (P < 0.05 for each). From the subsequent case-control study, we found that BMI, family history of malignancies and history of alcohol intake were associated with the development of HCC in PBC patients (P < 0.05 for each). In multivariable analysis, BMI (OR: 1.294; 95% CI, 1.054-1.589), and history of alcohol intake (OR: 9.204; 95% CI, 1.019-83.129) were significantly associated with increased odds of HCC. Conclusions: HCC is not rare in Chinese PBC patients. Moreover, the HCC incidence is higher and liver injury is more serious in male patients than that in female patients. BMI and history of alcohol intake are risk factors for HCC development in PBC patients. Therefore, PBC patients may benefit from abstinence of alcohol intake and control of body weight. Xue-Xiu Zhang, Li-Feng Wang, contributed equally to this study. *Correspondence to: Prof Fu-Sheng Wang, Research Center for Biological Therapy, fswang302@163.com, Beijing 302 Hospital, No. 100, the 4th Western Ring Middle Road, Beijing 100039, China.

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Possible impairment of mucosal-associated invariant T cells in the liver of patients with primary biliary cirrhosis

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BACKGROUND/AIM: Human mucosal-associated invariant T cells (MAIT) cells constitute a unique subset of innate-like T lymphocytes characterized by a semi-invariant T cell receptor (TCR) repertoire (made of an invariant Vα7.2-Jβ7.2-Jβ chain) capable of recognizing bacterial products. Although MAIT cells are abundant in the human gut and liver, the involvement of MAIT cells in the pathogenesis of liver diseases remains unclear. In consideration of the possible association between bacterial infections and primary biliary cirrhosis (PBC), we focused on the role of MAIT cells in PBC. METHODS: Heparinized peripheral blood and liver biopsy specimens were collected from 13 patients with PBC and 11 patients with chronic viral hepatitis (CVH). Surgically removed liver tissues distant from the tumor in 10 patients with metastatic liver tumors were used as control livers (Control). Mononuclear cells were separated by Ficoll-gradient, and then various surface markers were investigated by flow cytometry. mRNA expression was quantified by real-time PCR. Cytokine production was investigated using peripheral blood MAIT cells after stimulation with anti-CD3/CD28-coupled beads in the presence or absence of IL7. We also investigated the distribution of V7.2+ CD161+ cells in the liver by immunohistochemical staining. RESULTS: In the Controls, CD3+ TCR-CD161+ cells comprised 6.8% (median) (range 1.1-17.9%) of the total T cells in the liver but only 1.6% (0.1-6.7%) of the total T cells in the blood. Intrahepatic MAIT cells constituted a significantly lower proportion...
in PBC patients (1.9%, 0.7-8.8) than in CVH patients (8.9%, 0.2-20.7) and Controls. We found a significant decrease in the proportion of activated CD69+ MAIT cells in the liver of patients with PBC compared to patients with CVH and Controls. After the normalization of alkaline phosphatase by treatment with ursodeoxycholic acid, MAIT cells increased in the blood. Although MAIT cells express high levels of the IL-7 receptor (IL-7R), MAIT cells in the liver of patients with PBC expressed less IL-7R (66.8%, 60.0-70.5) than in the liver of patients with CVH (76.3%, 44.4-93.7) and Controls (89.1%, 38.5-94.8). We also confirmed that the functions of MAIT cells were dynamically regulated by the presence of IL-7.

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313 Circulating bile acids and sterol levels in patients with cholestatic pruritus. Effects of albumin dialysis using MARS
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Background and aims: Serum metabolomic profile and changes before and after treatment with albumin dialysis using the molecular adsorbents recirculating system (MARS) were assessed in patients with cholestatic pruritus to identify metabolites potentially associated with the pathogenesis of itch. Patients and Methods: Serum samples were obtained from 85 patients with primary biliary cirrhosis, 21 with pruritus (9 with resistant pruritus before MARS) and 64 without pruritus. Moreover, serum samples before and after MARS and albumin dialyze were taken in the 9 patients with resistant pruritus. Metabolite extraction was accomplished by fractionating the samples into pools of species with similar physicochemical properties, and three different platforms were used to perform optimal profiling of: a) fatty acids, bile acids, steroids and lysoglycerophospholipids; b) amino acids; and c) glycerolipids, sterol lipids, sphingolipids, and glycerophospholipids. The analyses were performed by UPLC-ESI-TOF-MS and multivariate and univariate analyses. Results: More than 470 metabolites were identified. Bile acids were significantly higher in patients with pruritus (p<0.001), particularly in those with resistant pruritus. Cortisol (p=0.02) and androsterone sulfate were in lower levels, while pregnenolone sulfate and an isomer of dehydroepiandrosterone sulfate (DHEAS) were higher in patients with pruritus. Most metabolites decreased in sera after MARS and the differences were particularly significant for sterols, N-acetyl ethanolamines, 1-ether, 2-acetylglycerophosphatealanine and free sphingoid bases. MARS treatment resulted in a significant reduction of primary bile acids (p = 0.03) and secondary bile acids, pregnenolone sulfate (p=0.007), DHEAS (p=0.02), an androsterone sulfate isomer (p=0.003), some glycerophospholipids and kynurenine (p=0.02). Four of these serum metabolites, including bile acids were identified in the albumin dialysate. Conclusion: Cholestatic pruritus is associated with increased bile acids and changes in the lipid profile. MARS therapy for pruritus results in a decrease of circulating metabolites especially phospholipids, primary bile acids and sterols. This metabolomic analysis identifies a panel of biomarkers that could participate into the pathogenesis of cholestatic pruritus.

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Albert Pares - Consulting: Lumena Pharmaceuticals

314 The neogenesis of high endothelial venules and the formation of tertiary lymphoid organs in primary biliary cirrhosis
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Purcose: Recently, it has been reported that the migration of inflammatory cells via high endothelial venules (HEVs) is related to the pathogenesis in various chronic inflammatory diseases. Moreover, it is known that inflammatory areas with HEVs sometimes show the characteristics of secondary lymphoid tissue, and these structures are called “tertiary lymphoid organs (TLOs)”. In the present study, to examine whether the neogenesis of HEVs and the formation of TLOs are occurred in primary biliary cirrhosis (PBC), we performed the histopathological study. Methods: We examined the liver specimens of 21 PBC cases, 13 chronic viral hepatitis-C (CVH) cases, and 5 normal cases. We performed immunohistochemistry for MECA-79, which is well-established marker of HEVs, CD3, CD20, CD21, CD83, and CCR-7. Results: In PBC livers, HEVs were observed more frequently in portal areas with lymphoid aggregation in PBC than in CVH (78% versus 27%; p < 0.01 Fisher’s exact test). On the other hand, HEVs were never observed in normal livers. In addition, CCR-7+ lymphocytes, which migrate to peripheral tissues via HEVs, were observed more frequently in inflammatory cites in PBC livers compared to CVH livers. Moreover, in PBC livers, HEVs were observed in 77% of portal areas with bile duct obstruction, whereas they were observed in 28% of portal areas without bile duct obstruction (p < 0.01 Fisher’s exact test). Next, we examined whether TLO’s features are recognized in 13 PBC cases, in which HEVs were remarkably observed. As a result, all these cases contained at least one inflammatory area with following features specific to lymphoid tissues; (1) follicular dendritic cells (FDCs) labeled by CD 21 were observed (2) B cells labeled by CD20 surrounded FDCs and formed germinal center (3) Distinct but adjacent T cell, labeled by CD3, and B cell components were recognized (4) Dendritic cells labeled by CD83 were observed in the margin of T cell area. Conclusions: We revealed the neogenesis of HEVs and the formation of TLOs in PBC livers. These phenomena can be related to the pathogenesis of PBC.

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315 Geoepidemiology of primary biliary cirrhosis in Central Greece
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Background and aims: Genetic and environmental factors have been implicated in primary biliary cirrhosis (PBC) pathogenesis. Our aim was to describe the epidemiological characteristics and the spatial distribution of PBC in Central Greece. Methods: The study was performed in Thessaly, one out of the thirteen regions of Greece, which covers most of the part of Central Greece. During the last 13 years, 281 PBC patients...
(253 females, 90%) residents of Thessaly region were appropriately diagnosed. Results: The mean±SD age of the patients during the initial presentation was 57±13 years. Anti-mitochondrial antibodies were detected in 93.2% of the patients, while 48.8% were asymptomatic. Among known risk factors, a history of urinary tract infection was reported in 6.4%, hormonal estrogen replacement in 1.4%, previous/active smoking in 24.9%, presence of other autoimmune disease in 21.7%, and family history of autoimmune disease in 7.5% (familial PBC in 4.3%). The median annual incidence was 23 new cases per year. The date of first manifestation of the disease could be identified in 99 patients, with a marked peak during the spring (P=0.01). The overall prevalence of PBC in Thessaly was 373 per 1 million inhabitants, which was not equally distributed. Six districts showed a prevalence >800 per 1 million inhabitants. Conclusion: There is an increased prevalence of PBC in Central Greece with remarkable geographic clustering. These data along with seasonal variability may suggest environmental risk factors in PBC pathogenesis.

316 Hepatic sarcoidosis: To treat or not to treat?

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Background: Despite recent advances in immunotherapy, data on the benefits of treatment of hepatic sarcoidosis are limited.

Aim: To compare the course and outcomes of patients treated for hepatic sarcoidosis with those of untreated patients.

Methods: Patients with hepatic sarcoidosis, diagnosed clinically, radiographically or histologically (ICD code 135) in the Liver Clinic of the University of Chicago from July 2000 to June 2012, were identified. Demographic, clinical, laboratory, histologic and treatment data were obtained and analyzed with the Stata software.

Results: 70 patients were included in the study, with a mean follow-up of 57±51 months. 28(40%) were males, with a mean age of 49±10 yrs. 80% were African Americans. 32% were asymptomatic, while 40% had gastrointestinal symptoms. The prominent liver test abnormality was an elevated alkaline phosphatase (AP) level (375±383 IU/L). Angiotensin-converting enzyme (ACE) levels were elevated only in 49% of tested patients (n=53). Of 55 patients who had a liver biopsy, 30(55%) had no fibrosis, 14(25%) had stage 1-2, and 9(16%) had stage 3-4. 13 patients (11 treated) had paired liver biopsies over a 59±38 mos interval; 6(46%, 1 untreated) showed no change, 6(46%, 1 untreated) showed improved fibrosis, while 2(15%) showed worse fibrosis at follow-up. 52(74%) patients were treated for sarcoidosis, 31(60%) with corticosteroids, 19(37%) with ursooxygenic acid, 18(35%) with other immunotherapy agents.

Demographic and baseline laboratory data and the follow-up periods were similar between the treated (TX) and the untreated (No TX) groups, except for a higher albumin in the No TX group (4.2±0.4 vs 3.9±0.5 g/dL, p=0.02) and a lower AP in the No TX group (224±207 vs 428±416 IU/L, p=0.05). Comparison of baseline and follow-up laboratory data for each group are shown in the table. 6% of patients in each group either died or required OLT. Conclusions: Liver chemistry tests may improve in hepatic sarcoidosis with or without therapy, although untreated patients had lower AP in the treated than in the untreated group. Transplant-free survival is similar in treated and untreated patients.

| Patient (KPa) | Untreated Group | Treated Group | p value | Baseline | Follow-up | p value |
|--------------|----------------|--------------|---------|---------|-----------|---------|
| Platelet K/Dl | 224±79         | 197±86       | 0.04    | 227±56  | 193±79    | 0.001   |
| INR          | 1.0±0.05       | 1.1±0.03     | 0.02    | 1.0±0.02| 1.0±0.03  | 0.23    |
| Creatinine (mg/dL) | 1.2±0.21 | 1.5±0.35    | 0.1     | 1.2±0.25| 1.3±0.38  | 0.15    |
| Albumin (g/dL) | 3.9±0.5        | 3.7±0.33     | 0.02    | 3.8±0.5 | 3.8±0.6   | 0.001   |
| ALT (U/L)    | 422±420        | 312±107     | 0.01    | 662±49 | 468±23   | 0.001   |
| AST (U/L)    | 542±23         | 312±107     | 0.01    | 716±26 | 454±28   | 0.01    |
| ALB (U/L)    | 542±23         | 312±107     | 0.01    | 716±26 | 454±28   | 0.01    |
| ACE (U/L)    | 712±68         | 342±241     | 0.17    | 787±56 | 592±45   | 0.11    |

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317 Age at Presentation and Gender are Predictors of Increased Mortality Over Long-Term Follow-Up in Primary Biliary Cirrhosis (PBC)

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Background: Recent findings from the prospective UK-PBC patient cohort have shown that non-response to ursodesoxycholic acid (UDCA) therapy is associated with increased risk of death or need for transplant in PBC. Younger age at presentation and male gender were associated with increased risk of UDCA non-response. Although the implication is that age at presentation and gender are therefore risk factors for death and transplantation in PBC, the link as yet, has only been an indirect one. Here we set out to utilise the historic Newcastle cohort to directly explore the impact of age at presentation and gender on outcomes in PBC. Aims: To utilise a large and comprehensive, geographically defined, long-term follow-up cohort of PBC patients and matched community controls to explore the impact of age at presentation and gender on actual outcome in PBC. Methods: Kaplan-Meier survival analysis in the comprehensive North-East England PBC patient cohort of 588 PBC patients (529 female) incident between 1979 and 2003, prior to the widespread use of UDCA in Newcastle. Cohort partic-

AASLD ABSTRACTS

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The FXR Agonist Obeticholic Acid (OCA) Improves Liver Biochemistry Parameters Correlated With Clinical Benefit Across a Range of Patient Characteristics

Pietro Andreone, Giuseppe Mazzella, Simone I. Strasser, Christopher L. Bowlus, Pietro Invernizzi, Joost Drenth, Paul J. Pocksos, Jaroslaw Regula, Michael Trauner, Annarosa Floreani, Simon Hohenseer, Velimir A. Luketic, Mitchell L. Shiffman, Karel J. van Erpecum, Victor Vargas, Catherine Vincent, Bettina E. Hansen, Frederik Nevens, Richard Pencek, Roya Hooshmand-Rad, Shawn Sheeron, David Shapiro, Pietro Inv, Victor Vargas, Catherine Vincent, Bettina E. Hansen.

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Background and aims: The Phase 3 POISE trial evaluated the efficacy and safety of obeticholic acid (OCA), a derivative of chenodeoxycholic acid and potent farnesoid-X receptor agonist, in patients with PBC. The primary endpoint was substantially reduced compared with controls matched for age at diagnosis (p<0.0001, HR 1.31 (95% CI 1.7-26.6)). Conclusions: Younger age at presentation and male gender are important factors in determining risk of death or need for transplant in PBC and should be included for models of stratified disease management.

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FXR Agonist Obeticholic Acid: Sustained Improvement in Markers of Cholestasis and Long-Term Safety in Patients with Primary Biliary Cirrhosis through 4 Years

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Obeticholic acid (OCA, 6-ethyl chenodeoxycholic acid) is a highly potent FXR agonist being developed for the treatment of primary biliary cirrhosis. Efficacy and safety of OCA, given as monotherapy, was evaluated in a 12-week, Phase 2, double-blind placebo controlled trial and showed significant improvement in alkaline phosphatase (ALP), bilirubin and other indices of cholestasis, inflammation and hepatic function. This was followed by an open-label long-term extension period in which patients either continued OCA or switched from placebo to OCA. This analysis evaluated the safety and efficacy of OCA through 3.5 and 4 years of treatment. During the ongoing open label extension period, subjects initiated OCA at 10mg once daily and titrated to 50mg based on response and tolerability. Ursodeoxycholic acid (UDCA) was added in 11 subjects. Subjects (N=28): mean age 58yrs; female: 78%; Caucasian: 96%. Baseline: ALP: 442±275U/L; bilirubin: 4.6±3.2mmol/L; GGT: 460±318U/L; ALT: 91±61U/L; AST: 72±39 U/L. Median exposure was 3.8 (2.6-4.2yrs). Nine subjects terminated early overall; 6 due to AEs, 4 of which were pruritus. The majority of patients received OCA doses ≤25mg. Improvement in serum liver biochemical tests through 4 years of treatment was observed. Pruritus, the most common AE, improved with long-term treatment; The incidence of new onset pruritus declined after the 1st year and severity tended to decrease with continued treatment. PBC is a chronic cholestatic liver disease with persistent significant unmet need. Long-term OCA treatment in this study maintained a durable improvement in ALP and other hepatic biochemistry analytes with no new safety findings and improved tolerability.

| ALP     | GGT     | ALT     | AST     | Bilirubin |
|---------|---------|---------|---------|-----------|
| % Change at 3.5 years (n=19) |
| -19.9 (16.9) | -41.4(17.6)* | -30.3(12.0)* | -18.8(10.3) | -12.5(7.4) |
| % Change at 4 years (n=15) |
| -14.7 (23.6) | -34.5(21.7)* | -31.6(12.1)* | -17.0(12.4) | -12.9(9.2) |

Data are mean(SE). *p≤0.05 change from baseline

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Secondary Sclerosing Cholangitis Following Major Burn Injury

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Secondary sclerosing cholangitis in critically ill patients (SSC-CIP) is a relatively new previously unrecognized entity which may lead to severe biliary disease with rapid progression to cirrhosis. It is possibly mediated by ischemic damage of the biliary tree, followed by bacterial colonization and progressive destruction of biliary ducts. SSC-CIP is described very rarely in patients following major burn. We present for the first time a case series of patients with rapidly progressive SSC-CIP requiring aggressive intensive care treatment following major burn injury. The radiologic, endoscopic, medical and surgical treatments as well as the clinical course and outcome of these patients were retrospectively analyzed and reviewed. SSC-CIP was diagnosed in 6 consecutive patients hospitalized due to major burn injuries at the Intensive Care Unit (ICU) of the Sheba Medical Center, during the period from January 2008 to August 2013. SSC-CIP was diagnosed when ERCP or MRCP demonstrated the typical appearance of irregular intrahepatic bile ducts with multiple strictures and dilatations and/or, when a liver biopsy demonstrated severe cholestasis and degenerative biliary epithelium. Patency of portal vein and hepatic artery was confirmed by abdominal ultrasonography with a Doppler study. All patients were males; none of them had recorded evidence of pre-existing liver disease. Ages varied from 18 to 56 years old. All patients suffered from severe (grade 2-3) burn injuries with Total Burn Surface Area ranging from 35% to 95%. Mean length of ICU hospitalization was 115 (38-192) days. All patients required mechanical ventilation (with a mean peak pressure of 10cmH2O) and the administration of catecholamines for hemodynamic stabilization. All patients demonstrated severe cholestasis with a median alkaline phosphatase level of 2113 IU/L (range, 1194-3032IU/L) and median total bilirubin level of 28.3 mg/dl (range, 16.0-40.7 mg/dl). The diagnosis of SSC-CIP was confirmed by ERCP in one patient, MRCP in 4 patients and in 2 patients by a liver biopsy. Five patients were treated with Ursodeoxycholic acid in dose of 15mg/kg. Two patients developed multiple hepatic abscesses that were drained and grew hospital acquired multiple resistant bacteria. Two patients underwent orthotopic liver transplantation. Three patients (50%) died. In conclusion, SSC-CIP following major burn injury is a rapidly progressive disease with a poor outcome. Awareness of this grave complication is needed for prompt diagnosis and considerations of a liver transplantation. The pathogenic mechanisms leading to this condition warrant further elucidation.

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Discrepant Expression of miR-139-5p Between Serum and Liver in Patients with Primary Biliary Cirrhosis, and Its Possible Cellular Origin

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Background: PBC is considered to be an autoimmune disease, although its pathogenesis remains unclear. MicroRNAs (miRNAs) are known to be involved in the pathogenesis of a variety of diseases. In a previous study, we found that patients with PBC had serum miRNA profiles distinct from those with other liver diseases. Moreover, we found that these miRNA profiles showed differences in serum among three subtypes of PBC: 1) gradual progressive type (G type), 2) portal hypertension type (PH type), and 3) hepatic failure type (HF type). Accordingly, we evaluated the expression of these miRNAs in both serum and liver tissue, and sought the possible cellular origin of the miRNAs. [Methods] Patients with each of the three PBC subtypes, and healthy subjects as a control, were enrolled (n=5, respectively). Total RNA was extracted from individual serum and a library was prepared. Circulating miRNAs were detected using an Illumina Genome Analyzer IIX. After mapping to the database (miRBase), these miRNAs sufficiently validated were further evaluated. Differences in the levels of miRNAs were also examined by the laser capture microdissection (LCM) using paraffin-embedded liver tissues. Areas containing hepatocytes and infiltrating lymphocytes were selectively dissected, and the cell-derived miRNAs were quantified using a digital PCR apparatus (QuantStudioTM 3D). The expressions of specific miRNAs were then further confirmed using in situ hybridization. [Results] Among a total of 1514 miRNAs obtained, 97 miRNAs were then further confirmed using in situ hybridization. Heat map demonstrated that the miRNA profiles of both the HF and PH types were clustered differently from those of the G type and controls. Especially, miR-139-5p was significantly over-expressed in both the PH and HF type. qRT-PCR using serum samples also confirmed these data from deep sequencing. Digital PCR using tissue samples demonstrated that the levels of lymphocyte-derived miR-139-5p were higher than those from hepatocytes. In situ hybridization also revealed a higher incidence of miR-139-5p positivity in lymphocytes exhibiting CNSDC. [Conclusion] Comprehensive analysis has demonstrated characteristic miRNA expression profiles among the subtypes of PBC, miR-139-5p being characteristically down-regulated in serum from progressive subtypes. Results obtained from liver samples suggested that infiltrating lymphocytes were the source of miR-139-5p, although the levels of expression did not reflect those in serum samples. Our present findings suggest the involvement of a specific miRNA, miR-139-5p, in the pathogenesis of PBC, and especially in progressive clinical subtypes.

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Patient Experience and Characteristics of Cholestatic Pruritus in the UK-PBC Research Cohort

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Introduction: Pruritus is a common problem in cholestatic liver diseases such as Primary Biliary Cirrhosis (PBC). Pruritus has negative impact on patient quality of life. There are limited studies on characteristics, patient reported experience of cholestatic itch and its treatment. Aim: To utilize the data from the UK-PBC Research Cohort: 1) to report the prevalence and severity of pruritus in patients with primary biliary cirrhosis (PBC), 2) to describe patient reported information on their experience of itch and anti-pruritic therapy they had received. Methodology: This was an observational cross-sectional study of PBC patients recruited into the UK-PBC Research Cohort in which pruritus has been characterized as follows: 1) Frequency of itch (never, rarely, occasionally, frequently, all the time); 2) Experience of itch since diagnosis based on the PBC-40 itch domain; 3) Intensity of worst itch since diagnosis and in the last 7 days, measured using a visual analogue scale (VAS) and a 0-10 grading scale (GS); 4) Treatment for itch since diagnosis of PBC. We defined persistent itch as pruritus occurring ‘frequently’ or ‘all the time’, and severe itch as persistent itch combined with PBC-40 itch score ≥10. Results: Data were available for 2705 PBC patients without a liver transplant. 1889 patients (69.8%) had experienced itch at some point in their illness. Of these, 880 (46.5%) had persistent itch and 428 (22.6%) had severe itch. Table 1 summarizes main results. Correlation between VAS and GS of itch intensity was statistically significant (Spearman’s correlation coefficient 0.95, p<0.01). Patients with severe itch had received the following treatments: colestyramine (217, 51%), rifampicin (70, 16.3%) and naltrexone (33, 7.7%). Notably, 193 (45%) patients with severe pruritus reported no anti-pruritic treatment at all. Conclusions: Our results highlight the prevalence of pruritus in PBC. In the UK-PBC cohort, nearly a third of patients had experienced itch, of whom approximately one-half had persistent itch and one-quarter had severe itch. However, it would seem that treatment of itch was unsatisfactory as many patients with severe pruritus did not receive any anti-pruritic therapy. Our results also suggest need for improvement in the awareness among clinicians for better management of cholestatic pruritus in PBC.

Table 1. Patient Experience and Treatment of Cholestatic Pruritus in the UK-PBC Cohort (n=2705)

| Itch category | Treatment received for Itch |
|---------------|-----------------------------|
|               | Colestyramine (n%) | Rifampicin (n%) | Naltrexone (n%) | Admission (n%) |
| Any experience of itch n=1889 (69.8%) | 472 (25) | 99 (5.2) | 42 (2.2) | 16 (0.8) |
| Persistent itch n=880 (46.5%) | 332 (37) | 89 (10) | 41 (4.6) | 14 (1.6) |
| Severe itch n=428 (22.6%) | 217 (51) | 70 (16.3) | 33 (7.7) | 11 (2.6) |
323 HLA-DR3 transgenic NOD mice, a novel model for autoantigen induced autoimmune hepatitis

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Autoimmune hepatitis (AIH) in humans is a severe inflammatory liver disease, characterized by interface hepatitis on histology and by the presence of circulating autoantibodies and hypergammaglobulinemia. There are two types of AIH, type-1 (AIH-1) and type-2 (AIH-2) characterized by distinct autoimmune serology. Patients with AIH-1 are positive for anti-smooth muscle and/or anti-nuclear (SMA/ANA) autoantibodies whereas patients with AIH-2 have anti-liver kidney microsomal type 1 (anti-LKM1) and/or anti-liver cytosol type 1 (anti-LC1) autoantibodies. Cytochrome P4502D6 (CYP2D6) is the anti-genic target of anti-LKM-1 and formiminotransferase cyclohydrolase (FTCD) the antigenic target of anti-LC1. AIH, both type 1 and type 2, is also linked to the Human Leukocyte Antigen (HLA) alleles -DR3, -DR4 and -DR7. Early animal models of AIH did not faithfully represent the human disease. We developed a novel mouse model of AIH using the HLA-DR3 transgenic mouse on the non-obese diabetic (NOD) background (DR3-NOD). Immunization of DR3-NOD mice with a DNA plasmid, coding for human CYP2D6/FTCD fusion protein, leads to a sustained elevation of alanine aminotransferase (ALT), development of ANA, and chronic immune cell infiltration and parenchymal fibrosis on liver histology. Immunized mice show an enhanced Th1 response and paucity of regulatory T cells (Treg) in the liver and a CYP2D6/FTCD specific T cell response in vitro. This new animal model will help in elucidating further the pathogenesis of AIH and in evaluating the efficacy and safety of immunoregulatory therapeutic interventions in vivo.

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Circulating macrophage activation markers, CD163 and CD206, are associated with disease severity and treatment response in patients with autoimmune hepatitis

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Introduction: Autoimmune hepatitis (AIH) is characterized by chronic inflammation and fibrosis. Soluble (s)CD163, a specific marker for activated macrophages, is a marker for disease activity, fibrosis, portal hypertension and prognosis in acute and chronic liver diseases. We hypothesized elevated sCD163 and sCD206 levels in AIH patients with acute disease activity and higher levels in non-responders than non-responders. Methods: We included 113 AIH patients (female/male 85/28, median age 50 (range: 17-79)), 93 with autoimmune hepatitis and 20 with overlap syndromes of AIH-PSC (n=7) and AIH-PBC (N=13). We measured sCD163 and sCD206 by ELISA and associated levels with parameters of disease activity and cirrhosis. Results: Soluble CD163 was significantly elevated in AIH patients with acute disease activity compared to AIH responders (6.96(3.3-15.4) vs. 1.62(0.80-3.24) mg/L). sCD163 levels correlated significantly with ALT (rho=0.47, P<0.001), IgG (rho=0.48, P<0.001), bilirubin (rho=0.30, P<0.001), alkaline phosphatase (rho=0.38, P<0.001), coagulation factors (II, VII, X) (rho=-0.30, P<0.01) and thrombocytes (rho=-0.24, P=0.014). There was no difference in sCD163 levels between the different groups of patients with or without cirrhosis at time of diagnosis. sCD206 showed a similar but less significant pattern. Conclusion: sCD163 and sCD206 levels were markedly elevated in patients with acute activity in AIH and were normalized in patients on anti-inflammatory treatment, even in patients with cirrhosis. Our data support significant macrophage activation in AIH and sCD163 may serve as a marker for treatment response of AIH patients.

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Can a dietary supplement induce autoimmune hepatitis?

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Purpose: The etiology of autoimmune hepatitis (AIH) is largely unknown, but xenobiotics, rare viruses and drugs like minocycline and nitrofurantoin have been implicated. With this report we want to bring attention to dietary supplements as a possible trigger for AIH. OxyElite Pro New Formulation (USPlabs, Dallas, Texas), a popular weight-loss herbal dietary supplement was linked to severe hepatotoxicity in 36 patients across the US. Our center has encountered 35 of these cases and seen most of them recover after discontinuation of OxyElite Pro. We now report a subgroup of patients that went on to developed AIH. Methods: Clinical data on demographics, drug use, laboratory studies, biopsies and outcomes were collected. We assessed causality and severity of liver injury according to Roussel Uclaf Causality Assessment Method/ Council for International Organizations of Medical Sciences (RUCAM/ CIOMS) scale and Drug-Induced Liver Injury Network (DILIN) method respectively. We assessed likelihood of AIH diagnosis using the Revised Original Scoring System of the International Autoimmune Hepatitis Group. Results: 35 patients with acute liver injury were identified at our medical center between May 2013 and January 2014. Two patients were transplanted, two died, 25 recovered. Six patients continued to have progressive worsening of liver function despite discontinuation of OxyElite Pro. Four out of six patients were hospitalized at initial presentation, all had liver biopsies. Histology was consistent with AIH and distinctly different from other patients with OxyElite Pro DILI. All six patients were treated with corticosteroids and entered remission thus strengthening the diagnosis of AIH. Use of the Revised Original Scoring System revealed 2 cases as definite and 3 cases as probable. Conclusions: We report six cases of AIH in the setting of DILI due to OxyElite Pro in a five month period (August 2013-January 2014) observed in a single center. We postulate that DILI due to OxyElite Pro has induced de novo AIH or unmasked preexisting, quiescent disease.

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Analysis of Liver Stiffness Measured by Transient Elastography in Autoimmune Hepatitis

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Backgrounds and aims: Previous studies evaluated the usefulness of non-invasive assessment of liver fibrosis in patients...
with autoimmune hepatitis (AIH) only as a part of chronic liver disease category. The aims of this study were to evaluate performance of transient elastography (TE) in AIH patients and to predict cut-off values of significant fibrosis, defined as stages III and IV fibrosis by META VIR score. Patients and methods: Sixty patients, diagnosed as AIH at Gangnam Severance Hospital between Jan 2008 and Feb 2014, were included in this study. Diagnosis was made based on the diagnostic criteria by ‘Revised Original Scoring System of the International Autoimmune Hepatitis Group (1999)’. TE was performed to measure liver stiffness (LS) between 1 and 3 month after the diagnosis was made when acute flare of hepatitis was relieved. Forty-seven patients had liver biopsy performed for staging of liver fibrosis. Results: Patients were female predominant (M:F = 6:41) and 15 patients (31.9%) had significant fibrosis. On univariate analysis, the variables associated with significant fibrosis detected by liver biopsy are ALP (P = 0.016), GGT (P = 0.008), INR (P = 0.021), LS (P = 0.003) and duration for AST normalization after initiation of treatment (P = 0.002). On multivariate analysis, LS (OR = 1.216, 1.012-1.462, 95% CI), and duration for AST normalization (OR = 1.025, 1.002-1.048, 95% CI) were independent variables associated with significant fibrosis. The cut-off of LS ≥ 9.1 kPa had 94.4% sensitivity and 100% specificity for predicting significant fibrosis. The cut-off of LS ≥ 10.4 kPa had 100% sensitivity and 100% specificity for liver cirrhosis. LS predicted significant fibrosis (P = 0.0002) and liver cirrhosis (P = 0.001) better than APRI in AIH by AUROC. Conclusions: Transient elastography was proved to be a simple, reliable and useful method for assessing significant liver fibrosis in autoimmune hepatitis.

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The IL-7/CD127 axis negatively modulates the function of regulatory T-cells in autoimmune hepatitis
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Background and aims: Autoimmune hepatitis (AIH) is associated with numerical and functional CD4+CD25+ regulatory T-cell (Treg) defects. Bona-fide Tregs are negative for CD127 chain of the IL7 receptor – normally expressed on activated T-cells. IL7 is known to impact Treg function and survival. The aim of the current study was to evaluate the extent of Treg activation in AIH and to explore the role of the IL7/CD127 axis in modulating Treg function. Methods: 44 ANA- SMA+ AIH patients and 20 healthy subjects (HS) were studied. T-cell phenotype, transcription factor and cytokine profile was determined by flow cytometry. Immunomagnetically-isolated CD4 T-cells were cultured with TGFβ and IL2 to favour Treg skewing; their phenotype and cytokine profile was assessed by flow cytometry 4 days later. CD127+ and CD127- cell ability to suppress was tested in a proliferation assay following co-culture with CD4+ target cells. Purified CD4+, CD127+ and CD127- cells were incubated in the absence or presence of IL7 or IL2 for 20 minutes and then assessed for phospho-STAT5 expression. Their proliferation in response to IL7 was assessed after 48 hours. Results: The frequency of CD127+ cells within undivided CD4+CD25+ Tregs was higher in patients than in HS. CD127+ cells from both groups displayed lower frequencies of FOXP3+ and CTLA4+ cells and higher proportions of Tbet+, RORC+, IFNγ+ and IL17+ lymphocytes than CD127- cells. When the CD127+ subset was analyzed, lower frequencies of IL10+ cells were noted in patients compared to HS. Exposure to Treg skewing conditions resulted in: 1) reduction of CD127 expression in AIH and 2) increase in the frequency of IL10+ cells within CD127- cells in HS. In both groups addition of CD127+ but not of CD127- cells, resulted in marked suppression of target cell proliferation, which was partially abrogated in the presence of anti-IL-10 monoclonal antibody. Exposure to IL7 did not change the expression of phospho-STAT5 in purified CD4+, CD127+ or CD127- cells, but it led to a significant increase in CD4+ and CD127+ cell proliferation, more evident in AIH. Exposure to IL2 increased phospho-STAT5 expression within CD127+, but not within the CD127- subset both in AIH and HS. Conclusion: CD127+ cells are more frequent within Tregs from AIH patients and display a pro-inflammatory phenotype. At variance with their CD127- counterpart, CD127+ cells exert poor suppression. In contrast to IL2, IL7 does not induce the expression of phospho-STAT5 and promotes the proliferation of CD4 effectors. Taken together, these data show that the IL7/CD127 axis negatively modulates the function of Tregs in patients with AIH.

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Down-regulation of hepcidin production in patients with autoimmune liver diseases
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Background and aims: Hepcidin is synthesized in the liver and plays a pivotal role in iron metabolism by controlling both intestinal iron absorption and iron release from macrophages. Chronic inflammation and iron overload up-regulate hepcidin synthesis in order to reduce plasma iron concentration, while anemia and hypoxia down-regulate the production of hepcidin in order to increase iron availability. We investigated herein the possible role of hepcidin in diverse chronic liver diseases. Methods: Serum hepcidin levels and liver hepcidin mRNA were quantified in 126 patients (52 males) with different chronic liver diseases: chronic HCV infection (n=21), chronic HBV infection (n=23), chronic cholestatic liver diseases [primary biliary cirrhosis and primary sclerosing cholangitis, PBC/PSC] (n=34), autoimmune hepatitis (AIH) (n=16) and non-alcoholic fatty liver disease (n=32). Hepcidin mRNA levels were determined by extraction of total RNA from liver biopsy specimens and real-time quantitative RT-PCR. Hepcidin was quantified in patients’ sera drawn at the biopsy day by ELISA. Results: Both hepatic mRNA and serum hepcidin levels were significantly lower in female patients (p<0.035 and p<0.021, respectively). Univariate analysis showed a positive correlation between hepcidin serum levels and hemoglobin (p<0.01) as well as albumin (p=0.03), while they were negatively associ-
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Prevalence, Natural History And Outcome Of Overlap Syndrome Versus Autoimmune Hepatitis In The Indian Continent
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BACKGROUND ANDAIMS: The prevalence and spectrum of autoimmune hepatitis (AIH) and overlap syndrome (OS) is known to vary in different geographic regions of the world. Both these diseases are strictly defined by the presence of at least two of the three recognized biochemical, serological, and histological criteria. We aimed at defining the two patient populations and their demographic, clinical, serological and eventual outcome in the Indian continent. PATIENTS AND METHODS: Patients admitted to our hospital in past 4 years were reviewed retrospectively. The diagnosis was confirmed using simplified AIH score and Paris criteria for AIH and OS respectively. RESULTS: Of the 7686 patients analysed, 254 (3.3%) patients were found to fulfill criteria for AIH and OS. Out of this, 174 (68.5%) were AIH and 80 (31.5%) were OS. Majority of the patients were females accounting for 71.3% (n=124) of AIH and 72.8% (n=58) of OS. Patients with OS were older (46y vs. 42y) and had higher bilirubin levels (median 3.4 g/dL, IQR 1.8-11.8) as compared to AIH (2.2 g/dL; 1.2-5.9). Standard autoimmune markers were comparable, though, atypical autoimmune marker such as p-ANCA was seen more in OS group (10.1%, n=8). Osteopenia, as measured by DEXA, was more severe in OS patients (21.7%, n=13), p=0.01. Liver stiffness (FibroScan®) was higher in patients with OS (median 28.4; IQR 17.3-43; p<0.001). Patients of OS predominantly presented with cirrhosis (72.5% (n=58) as compared to AIH 64.9% (n=113); p=0.08. At first presentation, 56.3% of OS patients had decompensation in contrast to 37.9% of AIH patients; p=0.03. Overall, patients with OS carried poorer prognosis, as 8.8% died as compared to 5.2% of AIH; p=0.18. The presence of ASMA in the titre of 1:40 or 1:80 was associated with higher incidence of decompensation among patient in both the groups (p=0.04). The presence of ANA in the titre of 1:40 or 1:80 was negatively associated with decompensation in patients of AIH, but not in case of OS (p=0.05). CONCLUSIONS: AIH and OS are not uncommon as chronic liver disease in the Indian continent. Patients with OS are older and present more often as cirrhosis with decompensation with a poor prognosis. Decompensation is more likely in patients who harbour ASMA (p=0.04). We propose that high suspicion in diagnosis and lower threshold in performing liver biopsy in seemingly non-classical AIH would yield early diagnosis and could improve survival benefit in this group.

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Prednisolone Changes mRNA and longIntergenic non-codingRNA (lincRNA) Expression Profiles to Suppress Autoimmunity in CD4+ T Cells of Autoimmune Hepatitis Type 1
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Background/Aim: Autoimmune hepatitis (AIH) type 1, often occurs in middle age females, is a progressive autoimmune liver disease of unknown pathogenesis. Under the prednisolone (PSL) treatment, the progression of disease is mild in almost patients. However, some patients resist the PSL treatment, and some patients who achieved the remission by the PSL treatment recurrent easily without the PSL treatment. Thus, we analyzed the dynamics of gene expression by PSL treatment including messengerRNA (mRNA), long intergenic non-codingRNA (lincRNA), and miRNA in CD4+ T cells to reveal the mechanisms of PSL treatment for AIH. Methods: Clinically and pathologically diagnosed 2 naive AIHs, 7 AIHs in remission, and 7 healthy controls, who agreed to provide samples with written informed consent, were enrolled in this study. This study was approved by the institutional review board. Total RNA was extracted from CD4+ T cells purified from peripheral blood. The comprehensive analysis of the genes were undergone using microarray with statistics (ANOVA). The data were classified by hierarchical clustering and were analyzed for the function bioinformatically using Gene ontology (GO) analysis and pathway analysis. Results: Microarray study showed that 2,957 mRNAs (p<0.05, fold change>1.5), 574 lincRNAs (p<0.05, fold change>1.5), and 17 miRNAs (p<0.05, fold change>1.2) were differentially expressed among 3 groups. By the hierarchical clustering, each group was clearly distinguished by mRNA and lincRNA expression, and their expression profiles were classified into 4 clusters. Interestingly the patterns of lincRNA cluster were in inverse correlation with those of mRNA cluster. Moreover, bioinformatics revealed that 4 clusters of mRNA expression profile had the independent function each other by GO and pathway analyses. Conclusions: The gene expression profile of AIH in remission was not only different from naive AIH, but also from healthy control, suggesting that the PSL treatment for AIH dose not lead CD4+ T cells to normal condition but changes the expression profile to suppress the autoimmunity. These findings may contribute to the development of better treatment strategies against AIH.

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332 Fast corticosteroid tapering and early fibrosis stages: important risk factors for type 1 autoimmune hepatitis relapse in Japan

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Background: Autoimmune hepatitis (AIH) sometimes relapses after immunosuppressive therapies are discontinued or sometimes even when they are still being administered. Furthermore, relapse often occurs in the absence of AIH risk factors. Aim: This study aimed to identify the frequency of relapse and to analyze the risk factors associated with relapse in type 1 AIH patients. Methods: Clinical characteristics and therapeutic processes were assessed from 146 type 1 AIH patients. Relapse was defined as serum ALT levels ≥60 IU/L after corticosteroid treatment and serum ALT normalization (<30 IU/L). The corticosteroid reduction rate (mg/week) was calculated using the following formula: reduction dose (initial corticosteroid dose (mg) - corticosteroid dose (mg) at ALT normalization) / duration of corticosteroid treatment from initiation until ALT normalization (week). Results: Relapse was identified in 44 (30.1%) type 1 AIH patients after alanine aminotransferase (ALT) level normalization. ALT levels significantly increased when corticosteroid treatment was initiated, and histological examination identified that fibrosis stages were not progressed in relapsed patients compared with that in sustained remission patients. There was no intergroup difference in the proportions of discontinued immunosuppressive therapies (13.6% vs. 7.8%, p = 0.277). Moreover, there were no intergroup differences in the proportions of concomitant medications such as Ursodeoxycholic acid or azathioprine at the time of ALT level normalization. However, both reduction dose and rate of corticosteroid taper until ALT normalization increased in relapsed patients compared with sustained remission patients. Particularly, in 129 patients who did not receive pulse therapy, the reduction rate of corticosteroid taper and early fibrosis stages were significantly increased in relapsed patients compared with those in sustained remission patients. Multivariate analysis revealed that reduction rate of corticosteroids and fibrosis stages were significantly associated with relapse. The both reduction dose and rate were positively correlated with initial corticosteroid dose, ALT, and total bilirubin, respectively. Conclusion: Early fibrosis stages at corticosteroid initiation and a corticosteroid taper rate until ALT normalization were important AIH relapse risk factors.

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333 Serum sterol levels indicate distorted cholesterol homeostasis in cirrhotic patients with primary biliary cirrhosis

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Background: New cholesterol derives from de novo synthesis and intestinal absorption. Serum cholesterol precursor (e.g., lathosterol, desmosterol) and plant sterol concentrations (e.g., sitosterol, campesterol) represent valid surrogate marker for cholesterol biosynthesis and intestinal absorption, respectively. Since chronic liver diseases affects cholesterol homeostasis, we systematically investigated serum sterol levels in patients with primary biliary cirrhosis (PBC) with and without liver cirrhosis. Patients and methods: Overall, we recruited 111 non-transplanted PBC patients (age 22 - 83 years, 101 females). In this cohort, a total of 30 individuals (27%) presented with liver cirrhosis at diagnosis. Serum concentrations of plant sterols, cholesterol and its precursors were measured by gas chromatography/mass spectrometry (GC/MS). Patients with results suggesting familial hypercholesterolemia or hyperphytosterolemia were excluded from subsequent analyses. Serum markers were compared between cirrhotic and non-cirrhotic patients with non-parametric tests. Results: PBC patients with liver cirrhosis demonstrate significantly higher sitosterol and campesterol concentrations than non-cirrhotic individuals (P = 0.0002 and P = 0.0067, respectively). Serum levels of lathosterol and desmosterol are lower in these patients (P = 0.0001 and P = 0.013, respectively), who display a trend to lower serum cholesterol concentrations (P = 0.064). In cirrhotic patients, we identified increased sitosterol:cholesterol and campesterol:cholesterol but decreased lathosterol:cholesterol ratios (all P < 0.0001). Overall, the ratios of phytoestrols to cholesterol precursors are significantly (all P > 0.0001) higher in patients with liver cirrhosis as compared to non-cirrhotic individuals. Discussion: PBC patients with liver cirrhosis are characterized by decreased cholesterol synthesis and increased sterol absorption as compared to non-cirrhotic individuals. Determination of serum sterols may improve clinical stratification of patients with PBC. Further studies are required to investigate the association between liver metabolism in PBC and systemic cholesterol homeostasis.

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334 Metabolic syndrome induces a more rapid progression of fibrosis evaluated with transient elastography in early primary biliary cirrhosis

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Background: Metabolic syndrome (MS) is a comorbidity, possibly associated to PBC in up to 30% of patients, which...
335 Fibrates improve liver tests in primary sclerosing cholangitis with incomplete biochemical response to ursodeoxycholic acid: update of a pilot study

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Background: In patients with primary sclerosing cholangitis (PSC), ursodeoxycholic acid (UDCA) has no proven benefit on survival but improves serum liver tests and surrogate markers of prognosis. In the absence of other medical treatment with proven efficacy, UDCA is widely used in European PSC patients. However newer therapies are obviously needed. Fibrates are PPAR agonists that exert anti-inflammatory properties in several experimental models of autoimmunity and small series have suggested their beneficial effect on liver biochemistry in primary biliary cirrhosis. In 2010, we reported improvement of liver tests under fenofibrate for 6 to 12 months in some PSC patients with an incomplete biochemical response to UDCA. Aim: To confirm the safety and efficacy of fibrates in a PSC population with extended number of patients and duration of therapy.

Methods: This retrospective single-center study included patients with PSC treated with fibrates (fenofibrate 200mg/d or bezafibrate 400 mg/d) for at least 6 months in addition to UDCA, after an incomplete biochemical response (ALP ≥ 1.5 ULN) to UDCA (15-20mg/kg/d for at least 1 year). Patients with associated liver diseases, especially autoimmune hepatitis, were not included. ANOVA and Wilcoxon tests were performed to compare serum liver biochemistries at baseline, 3, 6, 9, 12, 18, 24, 36 and 48 months. Results: Fifteen patients were included: 10 males, median age 51 years, 9 with inflammatory bowel disease, median liver stiffness = 12.7 kPa (corresponding to fibrosis ≥ F3). Median duration of treatment with fibrates was 17.5 months (6.7-60.8). Under treatment with fibrates, ALP, GGT and ALT decreased significantly (p = 0.0001, 0.02 and 0.02 respectively). Biochemical improvement occurred early and 55% patients had ALP ≤ 1.5 ULN at 3 months. Total bilirubin and albumin remained unchanged. Two patients with dominant biliary stenosis developed cholelithiasis. No serious adverse event related to fibrates occurred. Conclusion: This study confirms that addition of fibrates induces a significant biochemical improvement in PSC patients with incomplete response to UDCA. Further studies are warranted to investigate the potential clinical benefit of fibrates in this context.

Course of liver tests during treatment with fibrates

| M0 | M3 | M6 | M9 | M12 | M18 | M24 | M36 | M48 |
|----|----|----|----|-----|-----|-----|-----|-----|
| Median ALP | 3.1 | 1.5 | 1.7 | 1.4 | 1.7 | 2.1 | 2.1 | 1.9 | 0.9 |
| Median GGT | 7.7 | 7.6 | 6.9 | 5.3 | 5.7 | 4.9 | 5.0 | 5.8 | 3.5 |
| Median ALT | 2.9 | 2.1 | 2.3 | 2.5 | 2.2 | 2.0 | 1.5 | 1.2 | 0.8 |

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336 Related Factors for Treatment-Dependent Autoimmune Hepatitis

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More than 80% of patients with autoimmune hepatitis are good responders to conventional treatment with azathioprine and prednisone. A small proportion of patients flares during prednisone tapering (treatment-dependent patients) according to AASLD guideline. We aimed to define response-related parameters in patients with autoimmune hepatitis (AH). Methods: The patients with AH who were followed up for at least 6 months were included into the study. Treatment-dependency was defined as ALT elevation during prednisone tapering. Those remained in remission with maintenance dose of prednisolone and/or azathioprine were defined as good responder (GR). Demographic data of the patients, baseline blood test values and biopsy findings, treatment options, laboratory tests at 6 months of treatment, length of the time of ALT normalization, and prognostic changes in the disease were investigated as associated with treatment-dependency and response. The number of exacerbations during prednisolone tapering were investigated as well. Results Twenty five of 81 patients with AH were treatment dependent (TD). Mean age was 52±18 (M/F: 1/24) and 55±13 (M/F: 8/48) in TD and GR patients, respectively. Six of 25 TD patients had more than 3 times exac-
berations during prednisone tapering. The patients with >3 exacerbations were younger than those with <3 exacerbations (56.6±16 vs 37±1.5; p=0.02). ALT normalization within 6 months in 16 (69.6%) TD patients and in 46 (88.5%) GR patients (p=0.046). Maintenance dose of prednisolone was higher in TD patients (8.05±4.8 vs 4.98±2.2 mg/day; p=0.016) as expected. Duration of prednisone treatment was longer in TD patients (44±29 vs 27±22 months; p=0.013). Side effects (29% vs 8.3%) and dose reductions (43% vs 20%) of azathioprine were more common in TD patients (p=0.05). ALT, AST, GGT, globulin levels were higher in TD patients comparing to GR patients at 6th month of therapy (p<0.05). Anti smooth muscle antibody (ASMA) positivity was more common in TD patients with higher number of exacerbations (%80 vs %27.8; p=0.056). Liver disease progression was observed in 9 TD (36%) patients and in 8 (14%) GR patients during a median of 27 (6-168) months of follow up (p=0.027). Conclusions. Treatment dependent patients use higher dose of prednisolone with longer duration. Their biochemical remission is achieved later comparing to GR patients'. Azathioprine side effects or intolerance are important issues for treatment dependency. As TD patients have more progressive liver disease, other immunosuppressive drugs such as mycophenolate mofetil or cyclosporine should be tried.

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Successful ex-vivo normothermic oxygenated machine perfusion of human donor livers
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Introduction: Currently empirical criteria are used to determine usability of donor livers however they have a low predictive value and alternative methods to determine viability are desirable. This ongoing study aimed to assess the feasibility of using a normothermic liver perfusion protocol in human donor livers, rejected as unsuitable for transplantation, as a tool to assess whether they would in fact be viable for clinical use. Methods: Organ retrieval and cold perfusion were performed in a standardized fashion using University of Wisconsin solution. In addition, blood from the donor was collected as perfusion solution. The perfusion circuit consisted of a single centrifugal pump which circulated perfusate out of the inferior vena cava through an oxygenator / heat exchanger and then split into a pressure-controlled hepatic artery supply and gravity fed portal venous supply via a reservoir. Throughout the perfusion period of up to six hours there was continuous monitoring of haemodynamic parameters and blood, bile, liver and bile duct tissue samples were collected. Results: At the time of submission, one liver donated after cardiac death (DCD) and one donated after brain death (DBD) have been studied. Both livers were metabolically active throughout the perfusion period reflected by lactate clearance (peak lactate 9 and 8.16 mmol/L; 0.95 and 2.56 mmol/L at the end of perfusion), urea production (4.4 and 4 mmol/L at start of perfusion; 11 and 7.9 mmol/L at end of perfusion) and bile production. Liver histology obtained at the end of the perfusion period showed no evidence of hepato-cellular injury. However, there was extensive biliary injury in the DCD liver as reflected by epithelial cell loss and mucosal necrosis of both the left and right hepatic duct. Conclusion: This study shows that normothermic oxygenated machine perfusion is feasible from a technical perspective in donor liver currently deemed unsuitable for transplant. This continuing study is comparing the results of both DCD and DBD donor livers. The extensive degree of biliary damage in the DCD liver may underline the need to consider biliary tract integrity when assessing graft viability.

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Serum zinc levels are associated with decompenated liver function and reduced survival in patients awaiting liver transplantation
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Background and Aims: Zinc is an important trace element with catalytic and defensive functions. We assessed the impact of zinc deficiency in patients with end-stage liver disease (ESLD) awaiting liver transplantation. Methods: Serum zinc levels were measured at the time of evaluation for liver transplantation in ESLD patients (n = 265). Patients were dichotomized in two groups: low and normal zinc serum levels. Results: Median serum zinc levels were 8.59 μmol/l ± 3.1. Kaplan Meier analysis showed impaired time to hydropic decompensation, hepatic encephalopathy, spontaneous bacterial peritonitis and hepatorenal syndrome (all p < 0.000, respectively) for patients with reduced serum zinc levels. Serum zinc levels remained an independent risk factor for development of hepatic encephalopathy (OR = 0.82; 95% CI: 0.73-0.92; p = 0.001) and hepatorenal syndrome (OR = 0.79; 95% CI: 0.68-0.91; p = 0.001) when subjected to multivariate analysis. Furthermore, actuarial survival free of liver transplantation was reduced for patients with low serum zinc levels [low zinc: 22.2 months; 95% CI: 17.4–27.0 vs. normal zinc: 30.1 months; 95% CI: 25.5–35.0; p = 0.003]. Patients with primary sclerosing cholangitis (PSC) are particularly affected by reduced zinc levels [low zinc: 12.5 months ± 2.4; 95% CI: 7.7–17.2 vs. normal zinc: 39.1 months ± 4.7; 95% CI: 29.8–48.5] resulting in impaired survival (p = 0.001) while this was not the case for patients with viral liver disease (p = 0.294), alcoholic liver diseases (p = 0.545) or patients classified with other hepatic disorder (p = 0.087). In PSC patients, serum zinc levels remained an independent predictor of survival when subjected to multivariate analysis [OR = 0.80; 95% CI: 0.64-0.98; p = 0.038]. Conclusions: We were able to identify serum zinc levels as a predictor of reduced survival in ESLD patients, particularly in PSC patients. Whether zinc supplemen-