the presence of high risk varices (HRV), defined as grade II, III esophageal and gastric varices, in patients with compensated liver cirrhosis. The **aim** of the study was to determinate the utility of spleen stiffness (SS) values measured by 2D-SWE as non-invasive marker for prediction of HRV, in patients with compensated liver cirrhosis.
Method: A prospective study was performed in 55 subjects with compensated liver cirrhosis, who underwent both spleen stiffness measurements (SSM) with a 2D-SWE technique (General Electric LOGIQ E9 XD clear 2.0) and upper endoscopy in the same admission. Spleen stiffness was performed with the patient in supine position and the SWE evaluation box was placed in the middle of the spleen, avoiding large vessels. Reliable SSM were defined as the median value of 10 measurements acquired in a homogenous area and an interquartile range/median (IQR/M) <0.30. Compensated liver cirrhosis was diagnosed based on clinical, biological and elastographic criteria (FibroScan > 12, 5 kPa)
Results: We obtained reliable SSM in 53/55 subjects (96.4%). 28/55 (51%) subjects had HRV. The mean SS values for patients with HRV were significantly higher as compared to those with first grade or no varices (18.6 ± 4.3 kPa vs. 15.7 ± 2.8 kPa with p = 0.0056). The best SS cut-off value measured with 2D-SWE-GE for predicting the presence of HRV in our study group was: 17.2 kPa (AUROC-0.80; sensitivity-67.8%; specificity-92%; PPV-91%; NPV-71, 9%).
Conclusion: Using the SS cut-off value > 17.2 kPa obtained by means of 2D-SWE we can rule in the presence of HRV, with a positive predictive value of 91%.

THU-076

SPP1 gene knockout in human hepatic stellate cells decreased profibrogenic cytokines and collagen gene expression

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Background and aims: Secreted phosphoprotein-1 (SPP1) is a stress sensitive multifunctional cytokine involved in innate immunity, cell proliferation, invasion, and metastasis. It was observed that SPP1 promotes activation and transformation of resting hepatic stellate cells into myofibroblast like cells and drives collagen synthesis. The purpose of the study was to evaluate the role of SPP1 on activation and transformation of human hepatic stellate cells and subsequent production of profibrogenic cytokines and collagen gene expression.
Method: Primary human hepatic stellate cells were isolated from pieces of surgically resected human liver tissue after pronase and collagenase digestion and purified using density gradient centrifugation. The isolated and purified stellate cells were cultured with appropriate medium for 48 h. The cultures were then divided into two sets and one set was treated with SPP1 CRISPR guide RNA sequences that efficiently knockout SPP1 gene without significant binding of Cas9 elsewhere in the genome. The second set of cultures without SPP1 CRISPR gRNA treatment served as control. The cultures were maintained for another 7–10 days. The cells were fixed and stained for α–smooth muscle actin (α–SMA) to examine the activation of stellate cells. Western blotting was carried out for SPP1, PDGF-B, TGF-β1, and collagens type I and type III. qPCR was performed to quantify mRNA of α-SMA, and collagens type I and type III.
Results: Immunocytochemical staining showed that the isolated quiescent stellate cells were positive for glial fibrillary acidic protein (GFAP) and negative for α-smooth muscle actin (α-SMA). Western blotting demonstrated complete knockout of SPP1 after treatment with CRISPR/Cas9 gRNA. Knockdown of SPP1 in primary hepatic stellate cells prevented activation and transformation of over 50% hepatic stellate cells into myofibroblast like cells and depicted marked decrease in the staining intensity of α-SMA. Western blotting for PDGF-B, TGF-β1, and collagens type I and type III showed significant decrease in the protein levels in SPP1 knockout cells. qPCR demonstrated marked reduction in the expression of α-SMA and collagens type I and type III mRNA indicating decreased activation of stellate cells and reduced synthesis of collagens after deletion of SPP1 gene.
Conclusion: The results of the present study demonstrated that SPP1 regulates profibrogenic cytokines and activation of stellate cells contributing to the pathogenesis of hepatic fibrosis. Furthermore, blocking of SPP1 has potential therapeutic implications to arrest hepatic fibrosis and related events.

THU-077

Fibro-protective molecular and cellular mechanisms emerge during resolution from thioacetamide-induced fibrosis

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Background and aims: The reported dynamic nature of hepatic stellate cells (HSCs) during fibrosis and resolution highlights that little is known of the fate of their co-migratory partner, liver progenitor cells (LPCs). Manipulating the known co-regulatory relationship between LPCs and HSCs during specific phases of fibrosis to alter their dynamic phenotypes may represent a new therapeutic target. Here we investigate thioacetamide (TAA) pre-treatment and recovery in mice, and the role and mechanism of resulting cell populations in the fibro-dynamic microenvironment of the liver after reintroduction of TAA.
Method: Tissue and sera were collected from C57BL/6 mice of two study groups (n = 5–9 per group): (a) pre-treated; 6-week TAA (300 mg/L drinking water), 4-week recovery, 6-week TAA and (b) naïve; 10-week control diet and 6-week TAA. Liver injury and fibrosis was characterized by extensive biochemical, histological and transcriptional analysis. Inflammatory, LPC and HSC subpopulations were identified by immunofluorescent staining. Primary isolated HSCs from healthy, fibrotic, and recovering mouse liver were co-cultured with the clonal bipotential murine oval liver (BMOL) LPC line prior to qPCR analysis of fibrotic transcriptional regulation.
Results: Phenotypically distinct inactivated desmin^{+}, aSMA^{-}, GFAP^{-} HSCs and CK19^{+} LPCs were confirmed in livers of pre-treated mice at d0, but not in naïve mice by immunofluorescence. TAA treatment induced early CD45^{+} inflammatory cell proliferation in naive mice typical to this model, which was notably absent in pre-treated mice. Elevated serum ALT levels and profibrogenic (aSMA, TGFb1, Col1A1 and TIMP1) transcription during early TAA treatment indicated a severe damage response in naïve mice. Incomplete resolution of fibrosis in pre-treated mice remained static throughout subsequent treatment, whereas naïve mice progressed to cirrhosis after 6 weeks of TAA. Primary HSCs isolated from fibrotic mice exhibit significantly reduced aSMA, Col1A1 and TGFb1 expression after co-culture with BMOL progenitor cells, suggesting anti-fibrotic regulation of HSCs through LPCs in this setting.
Conclusion: Contrary to the carbon tetrachloride model used in other recovery studies, TAA pre-treatment stimulates compensatory mechanisms, moderating the effects of a subsequent hepatic insult. As revealed through direct HSC-LPC coculture, LPCs may play a role in anti-fibrotic regulation of HSCs and could represent a mechanistic key to stabilising fibrosis for therapeutic benefit in patients with recurrent fibrotic pathology.