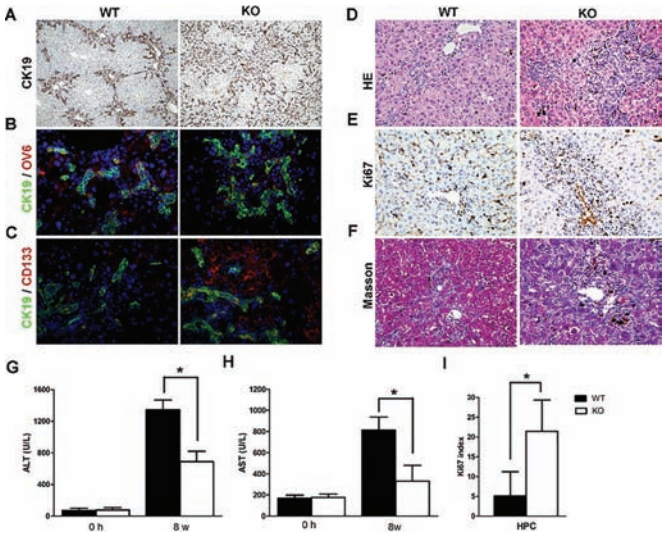
Their gene expression (acta, hsp47, and procollagenα1(I) (col1α1)) were measured by real-time qPCR.
Results: Efficacy of galunisertib was demonstrated by a concentration-dependent inhibition of fibrosis gene and protein markers. Galunisertib concentration-dependently inhibited col1α1 gene expression in human PCLS. Furthermore, the highest concentration of galunisertib significantly inhibited the expression of acta and hsp47 by 22 ± 9 and 55 ± 7%, respectively. Conversely, no significant impact was observed on their protein level. In rat PCLS, *acta, hsp47,* and *col1α1,* gene expression were concentration-dependently inhibited by 84 ± 4, 51 ± 5, and 92 ± 3%, respectively. Moreover, protein expression of α-SMA and HSP47 reduced by 74 ± 20 and 67 ± 7% in rat PCLS, respectively. The antifibrotic efficacy of galunisertib correlated with the inhibition of SMAD2 phosphorylation. Galunisertib inhibited SMAD2 phosphorylation by 84 ± 7 and 62 ± 12% in human and rat PCLS, respectively. In contrast, SMAD1 phosphorylation was upregulated by 612 and 185% in human and rat PCLS, respectively.
Conclusions: Galunisertib exhibits antifibrotic effects in both human and rat PCLS as illustrated by the inhibition of key fibrogenic genes and proteins. The blocking of TGF-βI receptor signaling via the inhibition of SMAD2 phosphorylation is the main mechanism of action of galunisertib. The activation of SMAD1 phosphorylation seems to be a compensatory mechanism of SMAD2 inhibition. However, SMAD1 upregulation appears to contribute little to fibrosis progression.

SAT-424
LOSS OF NUMB IN HEPATIC PROGENITOR CELL PROMOTES LIVER FIBROSIS IN MICE

X. Lu[1], L. Zhang[1], H. Ji[1], Y. Zhou[1], G. Guo[1], S. Luo[2], K. Zhu[1], X. Cao[1], Z. Wu[1], L. Li[1], H. Bu[3], Y. Shi[1]. [1]*Laboratory of Pathology, West China Hospital, Sichuan University, Chengdu;* [2]*Vasculocardiology Deparment, The Second Affiliated Hospital Of Chongqing Medical University, Chongqing;* [3]*Department of Pathology, West China Hospital, Sichuan University, Chengdu, China*
E-mail: 349753924@qq.com

Background and Aims: Hepatic progenitor cells (HPCs) contribute to differentiate into hepatocytes in severely injured liver with poor regenerative capacity. Inhibition of Notch signaling can prevent liver fibrosis in a bile duct ligation mouse model. The ubiquitin ligase Numb regulates the HPCs fate determinant through activation Notch signaling, but its role in liver repair remains unclear.



Methods: We crossed Numb floxed mice with Alb-Cre allele and fed Numb cKO mice with DDC diet to chronically activate HPCs. We performed immunohistochemistry to detect HPCs stem cell markers and proliferation. We also observed liver injury and liver fibrosis in DDC-induced liver by serum biochemical analysis and Masson's trichrome staining respectively.
Results: Disruption of Numb resulted in tremendous activation of HPCs concentrating around the portal triads and extending into the parenchyma upon DDC feeding. Notch signaling activation secondary to Numb deficiency did not affect the characteristics of HPCs as markered by OV6, CD133 and CK19. The disruption of Numb promoted HPCs proliferation and decreased DDC-induced liver injury, while deposition of extra cellular mesenchymal was significantly accelerated.
Conclusions: Loss of Numb robustly promotes the proliferation of HPCs in response to chronic liver damage, however, HPCs seemed differentiate into extra cellular mesenchymal producing cells rather than hepatocytes, therefore, promoted liver fibrosis.

Non-invasive markers of liver fibrosis

SAT-425
NEW CIRCULATING METABONOMIC AND MIRNOMIC BIOMARKERS TO PREDICT STEATOSIS, INFLAMMATION AND FIBROSIS SEVERITY IN NON-ALCOHOLIC FATTY LIVER DISEASE

A. Carretero[1], M. Lopez-Riera[1], E. Saez[2], T. Blazquez[2], I. Conde[1,2], A. Zaragoza[2], R. Jover[3], A. Lahoz[1]. [1]*Unidad de Hepatología Experimental;* [2]*Servicio de Medicina Digestiva;* [3]*Unidad de Hepatología Experimental-CIBERehd & Dep. Bioquímica y Biología Molecular, IIS-HOSPITAL LA FE, Valencia, Spain*
E-mail: agustin.lahoz@uv.es

Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is now the commonest liver disorder in the western world affecting up to a third of individuals. However, diagnosis is usually based on exclusion and liver biopsy is required for diagnosis. Therefore, finding new non-invasive biomarkers for accurate diagnosis and for discriminating among different stages of severity and progression has aroused much interest. Our objective has been to find new circulating biomarkers for steatosis, inflammation and fibrosis severity in NAFLD patients based on combined metabolomic and mirnomic approaches.
Methods: Observational and prospective study, which included 45 patients with NAFLD and compatible liver biopsy and 10 control patients, which underwent surgery for cholelithiasis but without NAFLD. Patients were classified according to the NASH CRN histological scoring system (NAFLD activity score -NAS) and the stage of fibrosis. Sample metabolite profiling was performed using a LC-MS approach that combines RP and HILIC chromatographic separations to ensure a high metaboloma coverage. MicroRNAs were quantified by real-time Q-PCR using specific forward primers and a universal reverse primer and TaqMan probe.
Results: 19% of patients were classified as not NASH (NAS_0-2), 26.2% as borderline (NAS_3-4) and 54.8% as NASH (NAS_5-8). 29% had grade 1 steatosis (6–33%), 27% grade 2 (34–66%) and 44% grade 3 (>66%). 33% had fibrosis stage F0, 16.7% stage F1, 14.3% F2, 26.2% F3 and 9.5% F4. The metabolomic analysis allow us to decipher a set of metabolites (mainly small peptides) that were able to distinguish between NAFLD and NASH patients, furthermore the metabolomic markers were also able to score the different degrees of fibrosis (F0-F4). The mirnomic analysis showed significant differences in the levels of: miR-663a (Control vs NAFLD, $p < 0.05$); miR-22-3p and miR-29a-3p (no steatosis & grade 1 vs grade 2–3, 0–33% vs >33%, $p < 0.01$). The serum level of miR-22 also showed significant differences between different degrees of NASH (Control vs NAS < 5 vs NAS ≥ 5, $p < 0.05$). Finally miR-22-3p was induced in patients with fibrosis ($p < 0.01$).

Conclusions: The combined use of metabolomic markers and miRNAs could facilitate noninvasive diagnosis of different stages of steatosis, inflammation and fibrosis in patients with NAFLD. Ongoing efforts are being dedicated to develop a model, based on these markers, aimed to score the severity of steatosis and fibrosis.
(Financial support: ISCIII/FIS 13/001470, ISCIII/PI14/00026).

SAT-426

CD49A−CD49B+ NK11 CELLS IN C57/BL FIBROTIC LIVERS ARE DISTINCT FROM PERIPHERAL NATURAL KILLER CELLS IN THEIR NEUROLOGIN4/CD107A ACTIVATORY MARKER

A. Salhab[1], J. Amer[1], R. Safadi[1]. [1]*Liver & Gastroenterology Units, Hadassah-Hebrew University Hospital, Jerusalem, Israel*
E-mail: johnnyamer@hotmail.com

Background and Aims: Natural killer (NK) cells are anti-tumor and anti-fibrotic protectors in the innate immunity. However, impaired NK cell killing reported in cirrhosis and some tumors. We found Neuroligin-4 (NLG4) gene expression on NK cells of liver injured patients from different etiologies were elevated including NAFLD and was associated with decreased in NK cytotoxicity. In this study, we aim to explore variations of NLG4 expressions on NK subpopulation (conventional NK cells CD49a− CD49b+) in liver and peripheral blood (PB) as a modulation of NK cells activity in fibrotic mice.
Methods: NK cells isolated from peripheral blood lymphocytes and livers of C57/BL induced fibrosis by Carbon-tetrachloride (CCl_4) injections (2X/wk for 2 weeks). NLG4 receptor expressions and NK activation marker (CD107, LAMP1-Lysosomal-associated membrane protein 1) on conventional populations assessed by CD49a−CD49b+ were determined by flow cytomerty technique.
Results: Liver NK cells expressing CD49a−CD49b+ were 17 ± 1.4% in naïve mice as compared to 36 ± 3.6% in fibrotic counterparts. PB NK cells expressing CD49a−CD49b+ were 27 ± 4.3% in naïve mice as compared to 30 ± 2.7% in fibrotic mice. While the liver NK cells of the naïve mice expressed 10 ± 1.2% NLG4 receptor, the NK cells of the fibrotic mice had significant elevations of NLG4 receptor (71 ± 9.1%; $p < 0.02$). These results were accompanied with a prominent decrease in % NK activation marker-CD107a in the fibrotic mice ($p < 0.01$). In parallel, PB NK cells from fibrotic mice had slight elevations in NLG4 receptor of 10 ± 9.1% as compared to the naïve mice (4.1 ± 0.6%) ($p = 0.04$). However, no elevations of PB NK NLG4 receptor were seen following CCl_4 injections.
Conclusions: Conventional NK cells although are dominated in the PB of mice as compared to livers showed to express low NLG4 receptors with no significant modulations in their functionality impairment and point to the role of liver conventional NK cells in attenuating/promoting liver fibrosis.

SAT-427

THE RELATIONSHIP BETWEEN FIB-4 INDEX VARIATIONS AND OUTCOME IN PATIENTS WITH CHRONIC HEPATITIS C AND CIRRHOSIS: COMPARISON WITH LIVER STIFFNESS MEASUREMENT

A. Vallet-Pichard[1], P. Sultanik[2], J.F. Meritet[3], S. Bouam[4], H. Fontaine[1], M. Corouge[1], A. Laurain[1], P. Sogni[1], S. Pol[1], V. Mallet[1]. [1]*Hepatology, Hôpital Cochin APHP;* [2]*Hepatology, Cochin APHP;* [3]*Virology;* [4]*Département Informatique, Hôpital Cochin APHP, Paris, France*
E-mail: anais.vallet-pichard@aphp.fr

Background and Aims: To evaluate the relation between FIB-4 index variations and clinical outcomes in a cohort of patients with chronic hepatitis C virus (HCV) infection and cirrhosis. The performances of sequential FIB-4 and liver stiffness measurements (LSM) were compared.
Methods: A cohort of 341 patients (male 66%; median age: 56 years) with HCV and proven compensated cirrhosis underwent sequential FIB-4 and LSM measurements from September 2006 to July 2015. Disease progression was scored as a composite end-point of end-stage liver disease (ESLD) and/or hepatocellular carcinoma (HCC). A mixed model analysis adapted for repeated measures and adjusted on gender, sustained virological response (SVR), HBsAg positivity, alcohol use disorders (AUD) and the metabolic syndrome was used to evaluate the relation between the annual FIB-4 and LSM variations and outcome.
Results: Overall, 14 (4%) patients had detectable HBsAg, 112 (33%) had AUD; 111 (32.6%) had the metabolic syndrome; 226 (66%) had received an interferon-based antiviral treatment and 45 (13%) had achieved SVR at first noninvasive test evaluation. The baseline median values of FIB-4 index and LSM were 4.26 (interquartile range [IQR], 2.28–8.36) and 16.3 (IQR, 10.2–27.5) kPa, respectively. After a median follow-up of 23.5 months (IQR, 11–51 months), disease progressed in 136 (40%) patients, including 57 (17%) with ESLD, 47 (14%) with HCC and 32 (9%) with ESLD and HCC. In a fully adjusted model, the risk of disease progression increased linearly with FIB-4 and LSM. A 2.7 unit increase or decrease of FIB-4 index or LSM increased or decreased the risk of disease progression by 14.5% ($p < 0.0001$) and 11.2% ($p = 0.007$), respectively. FIB-4 index and LSM variations were more closely related to ESLD than to HCC.
Conclusions: The variations of the inexpensive and readily available FIB-4 index, similarly to LSM, are related to disease progression, especially ESLD, in HCV-patients with cirrhosis, regardless of comorbidities and SVR.

SAT-428

USEFULNESS OF COMMON FIBROSIS MARKERS TO PREDICT VIREMIA IN PATIENTS WITH REACTIVE HEPATITIS C ANTIBODY TEST

A. De Weggheleire[1], S. An[2], J. Buyze[1], S. Thai[2], S. Francque[3,4], L. Lynen[1]. [1]*Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium;* [2]*Infectious Diseases, Sihanouk Hospital Center of Hope, Phnom Penh, Cambodia;* [3]*Gastroenterology Hepatology;* [4]*Laboratory of Experimental Medicine and Paediatrics, University of Antwerp, Antwerp, Belgium*
E-mail: adeweggheleire@itg.be

Background and Aims: Hepatitis C (HCV) diagnosis involves two steps: antibody screening followed by confirmatory molecular testing to "out-screen" resolved infections and biologically false-positives. Molecular testing is not widely available in resource-poor settings. We explored the added value of Aspartate-to-Platelet Ratio (APRI) and FIB-4 scores to overcome this bottleneck in the HCV diagnostic cascade.
Methods: We analyzed data from a cross-sectional study examining the prevalence of chronic HCV in a large HIV cohort in Phnom Penh, Cambodia. (*clinical trials.gov NCT02361541*). APRI and FIB-4 were calculated for patients with HCV IgG antibody positive or borderline result. ROC curves of APRI and FIB-4 to diagnose current coinfection (HCV IgG positive/borderlineand HCV-RNA positive) were established with corresponding area under curve (AUC) with 95% confidence interval (CI). Optimal cut-offs, chosen by weighing false negatives (FN) twice as harmful as false positives (FP), were defined as the point which minimizes *number of FP + 2* number of FN*. Sensitivity/Specificity was estimated with 95% CI for these cut-points. Finally the performance of the APRI/FIB-4 combination was assessed.
Results: By 03 November 2015, 2,326 patients were screened for HCV. Hundred eighty-three (7.9%) patients had a positive (N = 181) or borderline (N = 2) HCV IgG result; 79 tested HCV-RNA positive. Baseline parameters to calculate scores were available for all.
The AUC for diagnosis of chronic HCV was 0.78 (95% CI 0.71, 0.85) for APRI and 0.70 (CI 0.62, 0.78) for FIB-4. The optimal cut-points were 0.367 for APRI and 1.008 for FIB-4. The corresponding sensitivity was 81% (CI 70.6%, 89%) and specificity was 67.3% (CI 57.4%, 76.2%) for APRI; whilst being 79.7% (CI 69.2%, 88%) and 51.9% (CI 41.9%, 61.8%) for FIB-4.
Ninety-eight patients scored positive for HCV IgG and APRI > 0.367. Targeting HCV viral load to this subgroup would half the need for confirmatory testing. Fifteen diagnoses would have been missed; none of those had advanced fibrosis on transient elastography.