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ABSTRACT BOOK
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P01-01 Pilot study of liraglutide on weight and enzymes of obese patients with NAFLD

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a major risk factor for liver related morbidity and mortality. 30% of patients with NAFLD will progress to non-alcoholic steatohepatitis (NASH) that may lead to liver cirrhosis. NAFLD usually associated with obesity, type 2 diabetes, dyslipidemia and metabolic syndrome. Liraglutide is one of anti-obesity drugs that were approved by FDA. It is one of the glucagon-like peptide-1 receptor agonists (GLP-1RA). The aim of this study was to assess the efficacy and safety of liraglutide in the treatment of obese patients with NAFLD.

Methods: 26 obese patients with NAFLD were enrolled in this study who had BMI more than 30, randomized to receive either liraglutide 3mg subcutaneous daily for 4 months (13 patients) or placebo (13 patients).All patients were subjected to body composition analysis, muscle fat analysis, obesity analysis, segmental lean analysis and segmental fat analysis. In Body Score, waist-hip ratio and visceral fat level were fulfilled. Lipid profile, electrolyte profile, level of vitamin D, CBC, liver enzymes, abdominal ultrasound and thyroid gland ultrasound were obtained before and during treatment. BMI, In Body score and metabolic profile were assessed before, every 2weeks and after 4 months of treatment. Side effects were recorded every visit to determine safety of the drug.

Results: 4 months after treatment with liraglutide, there was reduction in body weight mean 75 ± 10.7 and there was significant reduction in ALT levels mean 17 ± 7 ($t = 5.8$ and $p < 0.001$) when compared to control group. The side effects were few such as nausea, decreased appetite and headache, with no significant difference between their occurrences in the two groups.

Conclusion: liraglutide is safe and effective in treatment of obese patients with NAFLD

P01-02YI RIP3-dependent signalling impacts on lipid metabolism and halts disease progression in experimental non-alcoholic fatty liver disease

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Background and aims: Necroptosis is activated downstream of death receptor stimulation and depends on receptor-interacting protein 3 (RIP3) kinase activity. Emerging evidence suggests important roles for specific lipids and lipid metabolism in necroptosis, as well as in NAFLD progression. Here, we aimed to evaluate the impact of RIP3 signalling in lipid metabolism, steatosis, inflammation, fibrosis and carcinogenesis associated with experimental NAFLD.

Method: C57BL/6 wild-type (WT) or RIP3-deficient (RIP3$^{-/-}$) mice were fed a choline-deficient L-amino acid-defined diet (CDAA; n = 14) or a control choline-sufficient L-amino acid-defined diet (CSAA; n = 14) for 32 and 66 weeks. Tissue samples were processed for histological and biochemical analysis of hepatic damage and carcinogenesis, insulin resistance and oxidative stress, and for lipidomic analysis.

Results: RIP3 deficiency ameliorated CDAA-induced inflammation and fibrosis and decreased the NAFLD activity score. In agreement, hepatic gene expression of pro-inflammatory mediators was also significantly decreased in CDAA-fed RIP3$^{-/-}$ mice, compared with WT, at both 32 and 66 weeks. Intriguingly, RIP3$^{-/-}$ mice displayed increased body weight gains, as well as increased liver fat accumulation at both time-points, compared with WT mice on the CSAA or CDAA diets. Lipidomic analysis showed that deletion of RIP3 shifted hepatic lipid species profiles. Particularly, RIP3 deficiency increased levels of diglycerides and triglycerides with shorter acyl chains and low unsaturation, while species with longer acyl chains and high number of double bonds were decreased in RIP3$^{-/-}$ comparing to WT under both CSAA and CDAA diets. Finally, RIP3$^{-/-}$ mice on the CDAA diet for 66 weeks tended to display reduced incidence of macroscopic preneoplasic nodules, accompanied by significantly reduced Ki67 positive hepatocytes. Indeed, microarray analysis and subsequent validation studies showed that the absence of RIP3 hampered the expression of oncogenes and signalling pathways controlling tumour microenvironment.

Conclusion: Overall, hepatic RIP3 impacts on lipid metabolism and plays an opposing role in controlling steatosis versus inflammation and carcinogenesis in CDAA-fed mice, leading to dissociation between these phenomena that are usually considered linked in NAFLD.

P01-03YI Co-creating comics to communicate non-alcoholic fatty liver disease - A participatory research approach

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Background and aims: The metabolic form of Non-Alcoholic Fatty Liver Disease (NAFLD) is driven by daily-life behaviors, namely hypercaloric diets and sedentary lifestyles, constituting a growing public health threat. Disease prevention/health promotion appeals for a behavioral change through health communication strategies. In a quest for effectivity, these strategies have shifted from a deficit model and embraced participatory approaches, allowing for a better integration of the plurality of knowledges that characterize the multifaceted nature of human behavior. Comics are proven science and public health communication tools, eliciting knowledge acquisition and attitude shifts. The inherent narrative format is particularly suited to convey biomedical knowledge whilst embedding individual and macro-social dimensions in the comics' world and the characters' journeys.

Method: Addressed via participatory research, our project involves the co-creation and evaluation of a comic that contextualizes NAFLD biomedical information to the non-biomedical perspectives of the disease. To our knowledge this is the first qualitative study on NAFLD awareness, documenting the factual and tacit NAFLD knowledge of Type 2 Diabetes patients. Semi-structured interviews (N = 30) were audiotaped, transcribed verbatim and analyzed with MAXQDA2018 software package. Targeting knowledge gaps we are creating narrative drawings focused on the main emerging themes identified.

Results: NAFLD Awareness is low and metabolic knowledge is lacking in our cohort. Alcoholic cirrhosis (and non-alcoholic cirrhosis) awareness was prevalent, but knowledge on the progressive nature of fatty liver into cirrhosis was less common. Based on health promotion models such as the Health Belief Model or the Information-Motivation-Behavior model, higher awareness on mechanisms and progression of NAFLD is expected to result in more engagement on primary and secondary prevention strategies. The concepts of insulin resistance, NAFLD progression and nutrition knowledge are being explored in visual form in the process of construction of the comic.

Conclusion: This communication presents a critical overview to the experimental design and preliminary results of the initial stages of this research project. Future quantitative analysis of pre/post-questionnaires will determine the effectiveness of the comic to communicate biomedical concepts in the context of NAFLD and promote the adoption of healthy lifestyles.

Figure:
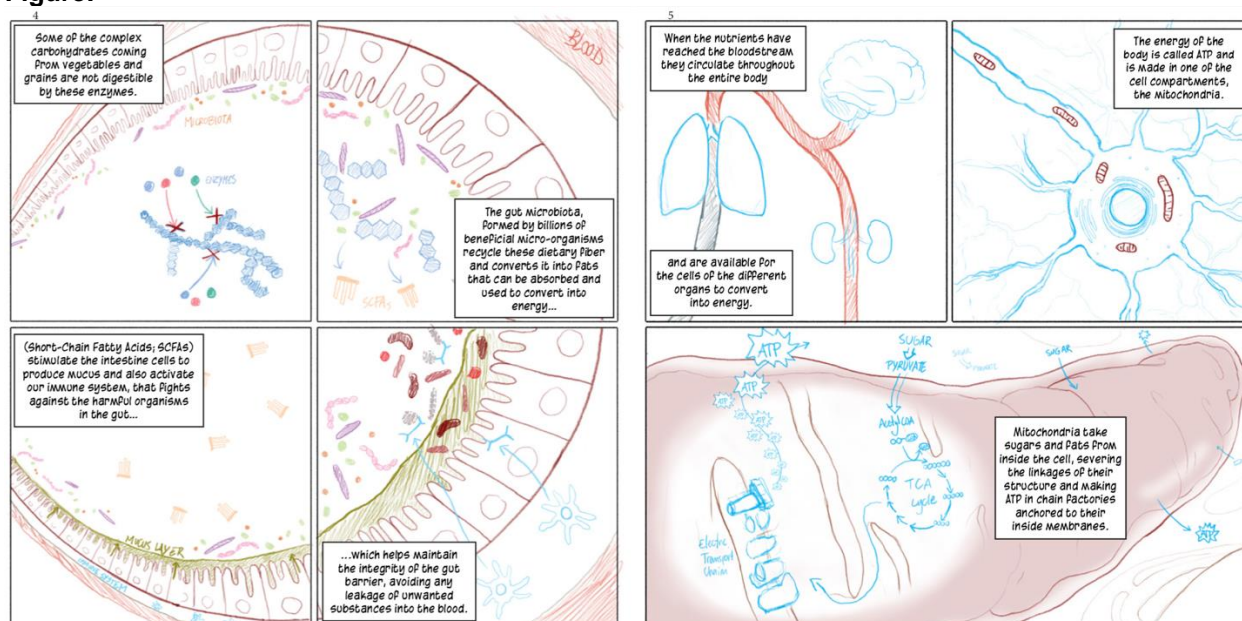
P01-04YI Decreased expressions of p70S6K in NK cells of NAFLD patients inhibited F-actin and was correlated with their impaired function

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Background and aims: Co-localization of p70S6K and stress fibers was suggested to regulate actin polymerization as rapamycin treatment could inhibit the elongation and organization of actin stress fibers via inhibition of p70S6K. We investigated effects of insulin resistance on mTOR signaling pathway of NK cells as a potential cause for their impairment in Non-alcoholic-Fatty Liver-Disease (NAFLD).

Method: Fresh peripheral blood NK cells isolated from healthy volunteers and 72 NAFLD patients (histology documented adults lacking full criteria of metabolic-syndrome), and characterized by flow-cytometry.

Results: Histologic progression of liver injury significantly correlated with elevated pro-inflammatory serum cytokines and insulin resistance. Western blot analysis of NK cells form NAFLD patients with F4 fibrosis showed to have dramatically reduction in PI3K pathway. ERK/MAP kinase pathway showed also reductions in these patients. Notably, these results were correlated with inhibitions in mTOR, p70S6K and F-actin phosphorylation ($p = 0.001$). NK stimulations with insulin (physiologic levels) reversed these effects. Compared to normal HOMA NK-cells by *in-vitro* co-culture with HSCs, high-HOMA CD56dim cells (with F3-F4) exhibited increased apoptosis and fail to block HSCs activation. While insulin incubation stimulated NK cell activation and killing of HSCs. Rapamycin reduced CD56dim expressions of insulin receptors (mimicking NAFLD insulin resistance) and prevented the insulin stimulation effect on NK cells.

Conclusion: Systemic Insulin-Resistance in NAFLD also includes the NK cells with reduced expressions of p70S6K and F-actin and therefore impairment in their function, which leads to cirrhosis and probably cancer.

P01-05 High prevalence of fatty liver in diabetes: comparison between ecographic and biochemical diagnostic methods

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Background and aims: Non-alcoholic fatty liver (NAFLD) is a condition determined by the deposition of excessive fat in the liver. This pathology gives rise to an inflammatory state (steatosis), which can progress to fibrosis, cirrhosis, or even liver cancer. NAFLD has been described as a frequent comorbidity of diabetes, and may also contribute to its genesis. In the case of people with diabetes, the presence of NAFLD may complicate management of glycemic control. Despite its importance, the evaluation of the presence and severity of NAFLD remains inconsistent, depending largely on the diagnostic method used.

This study`s aim was to determine the prevalence of NAFLD in a population of people with diabetes, comparing the evaluations of steatosis and fibrosis obtained by ultrasound and biometric/biochemical methods.

Method: People with diabetes, over 18 years of age, were recruited for an observational study, by convenience sampling, carried out at the clinic of the Portuguese Diabetes Association (APDP). Biometric and biochemical data was collected, and the Fatty Liver Index (FLI) and Fibrosis Index (FI) were calculated to assess diagnosis and severity. The evaluation was also performed by transient hepatic elastography through the Fibroscan® device, with internal algorithms for calculating steatosis and fibrosis.

Results: 690 people with diabetes were included in the study, 80% of whom were type 2 and 11% type 1 diabetes. The study population presented a prevalence of hepatic steatosis of 65% when assessed by elastography and 57% using FLI. Regarding the detection of fibrosis, this occurred in 45% of the individuals by elastography and in 19% by the FI. The level of agreement between the two methods was 74% for the diagnosis of hepatic steatosis and 22% for the detection of fibrosis.

Conclusion: The population with diabetes presented a high prevalence and severity of fatty liver. The ultrasound method showed great concordance in the evaluation of steatosis and greater apparent ability in the detection of fibrosis. This study supports the need to assess NAFLD presence in people with diabetes, in order to identify a group of patients with greater difficulties in metabolic control.

P01-06 High risk of non-alcoholic fatty liver disease and significant liver fibrosis in patients with hidradenitis suppurativa

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Background and aims: Hidradenitis suppurativa (HS) is a chronic inflammatory skin condition tightly associated to obesity and metabolic syndrome. The aim of this study was to determine the prevalence and risk factors of NAFLD in HS patients.

Method: this was a cross sectional, case control study including 70 consecutive HS patients above 18 years old that attended the Dermatology outpatient consultation of the University Hospital Marques de Valdecilla in Santander (Spain). Controls were age and gender matched in a 1:2 ratio and came from a general population random sample. NAFLD was established by liver ultrasound and controlled attenuation parameter (CAP) measured with transient elastography (TE). Liver fibrosis was estimated by liver stiffness measurement (LSM) with TE. Liver biopsy was obtained when significant fibrosis suspected.

Results: NAFLD prevalence was significantly higher in HS patients (51/70; 72.86%) compared to controls (37/150; 24.66%). In a multivariate logistic regression analysis, HS appeared as the strongest risk factor for NAFLD, independent from classic metabolic risk factors (adjusted OR: 7, 752). LSM was significantly higher in HS compared to controls, and in NAFLD associated to HS compared to NAFLD in control group. Among HS patients with NAFLD we found a high proportion of cases with significant (LSM>7, 2 kPa: 11/51; 21, 56%) and advanced (LSM>8, 7 kPa: 6/51; 11, 76%) liver fibrosis. In the multivariate logistic analysis stratified by gender, female patients with HS were at the highest risk of LSM above 6, 8 kPa, independently of classic metabolic risk factors.

Conclusion: NAFLD prevalence is significantly increased among patients with HS, with a high proportion of patients with advanced liver fibrosis. HS is the strongest risk factor for NAFLD, independently of classic metabolic factors. At the light of these results, the awareness of a concomitant presence of liver disease in HS patients should be encouraged, especially in the case of female patients.

Figure:
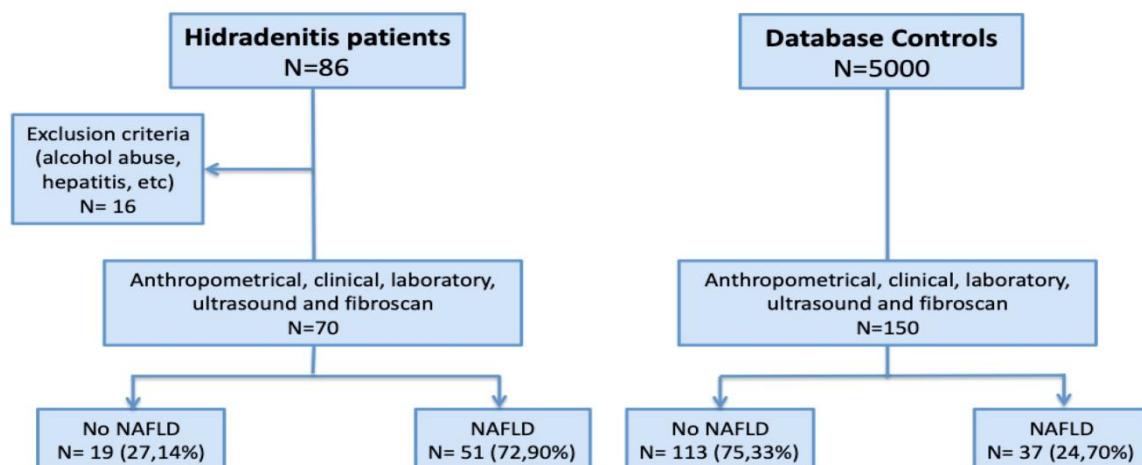
P01-07YI Hyperferritinemia and long-term outcomes in NAFLD patients. A longitudinal multicenter study

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Background and aims: Increased serum ferritin is commonly found in NAFLD patients and correlates with the severity of liver fibrosis. However, it's unclear whether it can predict liver events or mortality. Our aim was to assess the impact of ferritin levels on fibrosis, long-term outcomes and survival in NAFLD.

Method: We included 958 patients with biopsy proven NAFLD in tertiary centers from Italy (Turin, Milan, Rome, Palermo), Australia (Sydney) and UK (Newcastle). Clinical and biochemical data, including serum ferritin levels, were collected at baseline. Outcomes were collected for a median follow-up period of 90 months.

Results: Demographic data showed a median age of 47 [15-78] and a median BMI of 28.6 Kg/m2[17.8-58.8]). Histologically, 634 subjects had NASH while 732 had ≤F2 fibrosis and 221 had advanced fibrosis (F3-4). After a median follow-up of 90 months, 78 patients (8.1%) presented signs of liver disease progression (including ascites, encephalopathy, variceal bleeding); 17 (1.8%) developed HCC; 88 (9.2%) had cardiac events, 80 (8.4%) developed extra-hepatic cancer and 22 (2.3%) died.

Ferritin values were significantly different in F02 patients when compared to patients with F3-4 fibrosis, with higher levels in the latter (p = 0.003) and a small decrease in F4 patients. On the other hand, ferritin level difference between NASH and non-NASH patients was not significant. Notably, diabetic patients reported a trend to higher levels of serum ferritin (p = 0.052).

At univariate analysis, ferritin values >400 significantly predicted the prevalence of advanced fibrosis [OR 1.8 (1.2-2.6); p = 0.001]. This evidence was confirmed at multivariate analysis (including age, sex, BMI and diabetes) [OR 2.12 (1.4-3.2); p < 0.001].

Finally, patients with ferritin values >400 had worse survival at univariate analyses (log-rank 0.033), but in the Cox regression multivariate model only diabetes was found to independently predict mortality [OR 2.7 (1.1-7.1); p < 0.001] (Figure 1). No correlation was found between high ferritin level and liver events (including HCC).

Conclusion: Increased serum ferritin correlates with more severe liver fibrosis in NAFLD patients. However, this longitudinal study showed that high ferritin levels were not able to predict long-term outcomes. On the other hand, diabetes is the strongest and independent predictor of all-cause mortality.

Figure:

Funzioni di sopravvivenza
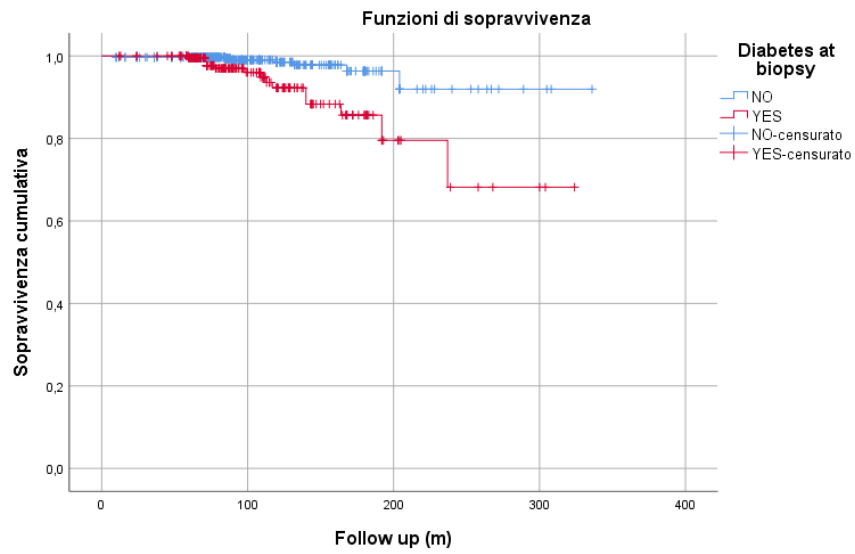
P01-08YI High fat-high sucrose diet promotes liver disease progression and defective metabolic adaptation

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Background and aims: Obesity is a chronic progressive disease associated to the main causes of mortality and comorbidity. Their prevalence has dramatically increased in the last decades, increasing the risk of suffer many metabolic diseases. Specifically, non-alcoholic steatohepatitis (NASH) the more severe form of non-alcoholic fatty liver disease (NAFLD) is accompanied by inflammation and hepatocyte damage and represents the main leading cause for liver transplantation. It's widely agreed that several factors may contribute to obesity and related metabolic liver disease but the alterations of nutrient inputs may be a potential contributor. The liver as metabolic organ is involved in the regulation of whole-body energy homeostasis and is metabolically flexible and able to readily switch the substrate fuel and store or mobilize nutrients according to metabolic needs. However, an inadequate response to metabolic challenge may lead to metabolic harmful effects.

Method: 5-weeks-old male mice (C57BL/6J) were fed with High Fat-High Sucrose diet (HF-HSD), High Fat Diet (HFD) or Standard Diet (CD) for 20 weeks (n = 8 per group). After this period, we determined liver status through histological analysis. To better understand of metabolic flexibility, we performed target metabolomics assays of liver energy metabolism and we evaluated AMPK/mTOR axis and proteins key in the regulation of fatty acid biosynthesis and oxidation.

Results: HF-HSD-fed mice was associated with clear alterations of hepatic metabolism, accompanied by steatosis, ballooning, lobular inflammation and lower hepatic antioxidant activity. These histological changes were comparable to NASH and our metabolic data revealed profound alterations at level of oxidative metabolism. In this context, we observed a decrease of some metabolites involved mainly in the glycolysis and tricarboxylic acid cycle (TCA), that may suggest a poor metabolic adaptation. In concordance with these alterations, we observed an inhibition of fatty acid biosynthesis and oxidation due to ACC1/ACC2 activity alterations in an AMPK-independent manner.

Conclusion: We report that HF-HSD-fed mice present important metabolic alterations that promote the liver disease progression observed in NASH.

Figure:
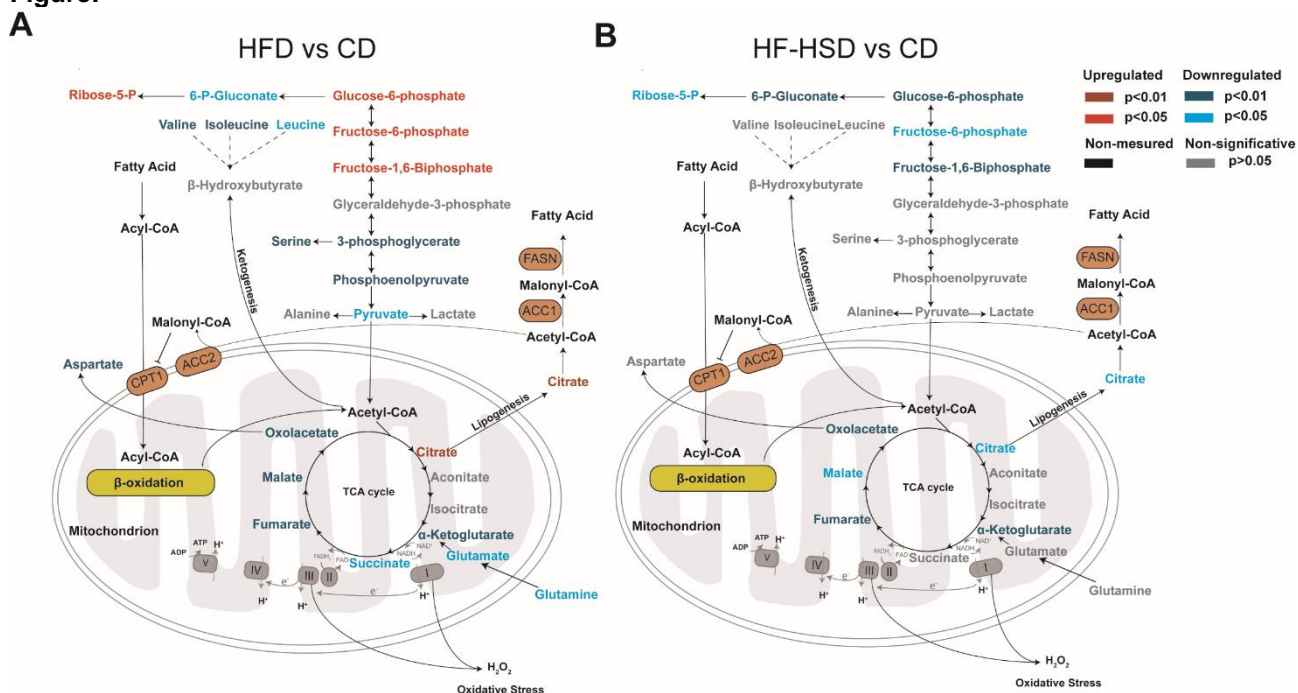
P01-09YI Liver fibrosis but not PNPLA3 mutation is associated with decreased renal function in non-alcoholic fatty liver disease

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Background and aims: Previous studies demonstrated an association between non-alcoholic fatty liver disease (NAFLD) and chronic kidney disease (CKD). Recent studies have focused attention on the role of PNPLA3 rs738409 polymorphism (the major genetic determinant of the susceptibility to NAFLD) in influencing renal function in adults and children with NAFLD. However, the association between PNPLA3 and CKD in NAFLD is still matter of debate. Aim of the study was to investigate the association between PNPLA3 rs738409 and kidney function in patients with NAFLD

Method: We studied 385 Caucasian patients with NAFLD and Metabolic Syndrome, consecutively attending the outpatient clinic of metabolic diseases Department of Internal Medicine and Medical Specialties of Sapienza University of Rome. NAFLD was evaluated by ultrasonography and metabolic syndrome was defined according with ATPIII modified criteria. Glomerular filtration rate (GFR) was estimated using MDRD equation. Fibrosis 4 score (FIB4) was calculated to estimate the amount of scarring in the liver. A FIB4<1.30 was considered consistent with the absence of liver fibrosis. PNPLA3 genotyping was performed by Sanger analysis. Multivariable logistic regression analysis was performed to calculate the adjusted Odds ratio (OR) and 95% confidence interval (CI) of factors associated with eGFR<90 ml/min/1.73 m2. In addition, a linear regression model was performed to investigate factor associate to eGFR.

Results: In the whole cohort, 52.2% of patients carried PNPLA3 rs738409 polymorphism. Mean age was 57.2 ± 10.8 years and 40% were women. Mean eGFR was 83.8 ± 18.8 ml/min/1.73 m2 and 67.2% of patients had an eGFR <90 ml/min/1.73 m2. No differences in eGFR were observed between rs738409 carriers and wild type patients ($p = 0.975$). Patients with FIB4<1.30 had higher eGFR (85.1 ± 19.0 vs. 80.6 ± 18.3 ml/min/1.73m2, $p = 0.028$) and the prevalence of patient with FIB4<1.30 progressively increase among eGFR tertiles (See Figure, $p = 0.012$). High waist circumference (OR = 0.26, 95%CI 0.07-0.91; $p = 0.03$) and FIB4<1, 30 (OR = 0.57, 95%CI 0.33-0.99; $p = 0.04$) were associated with eGFR<90 ml/min/1.73 m2 after correction for high triglycerides, PNPLA3 mutation, low HDL, high blood glucose, high blood pressure, hyperuricemia, high HOMA-IR and prior cardiovascular events., In addition, at multivariate linear regression, uric acid (Beta = -0.14, $p = 0.002$), Fib4 <1.30 (Beta = 0.12, $p = 0.035$), and high blood glucose (Beta = 0.16, $p = 0.012$) were independently associated with eGFR.

Conclusion: In our cohort of metabolic patients with NAFLD, PNPLA3 rs738409 polymorphism was not associated with decreased eGFR. By contrast, FIB4<1.30 was significantly related with lower eGFR values, suggesting that the severity of liver damage may independently predict kidney deterioration.
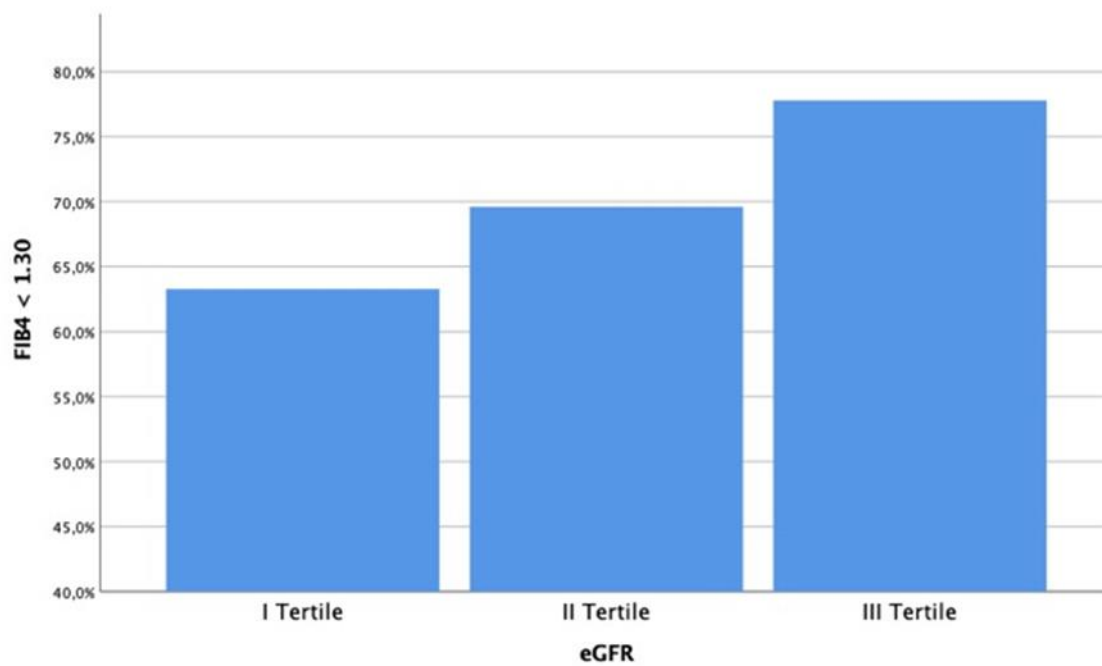
P01-10YI Plasma glycine concentration is inversely associated to hepatic and anabolic insulin resistance in subjects with NAFLD

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Background and aims: Altered amino acid (AA) profile has been shown to be a potential signature of metabolic diseases in particular non-alcoholic fatty liver (NAFLD). AA are the main substrate of muscle proteins and several studies have shown that insulin resistance (IR) is associated to increased protein catabolism/sarcopenia with net release of AA. Moreover, high plasma branched chain amino acid (BCAA) and low glycine concentrations were associated with IR and increased risk of NAFLD and diabetes (T2D). However, if lean and obese NAFLD show a different AA profile is still unknown.

The goal of this study was to evaluate 1) which AA concentrations were altered and 2) the association with hepatic, peripheral and adipose tissue IR in non-obese, nonOb_NAFLD, vs obese, Ob_NAFLD subjects.

Method: We studied 104 non_diabetic subjects, 41 obese (Ob) Ob_NAFLD, 44 nonOb_NAFLD (diagnosed by liver biopsy or ultrasound) and 19 non obese non NAFLD (CT). Fasting AA profile was measured by GCMS. IR was evaluated as HOMA (glucose*ins/22.5), hepatic IR (Hep-IR = EGP*ins) and adipose tissue IR (Lipo-IR = Raglycerol*ins), where the endogenous glucose production (EGP) and lipolysis (Ra-glycerol) were measured by tracer infusion. Anabolic resistance (AA-IR) was calculated as BCAA*INS, since fasting BCAA derive only from protein breakdown.

Results: Obese NAFLD had increased Hep-IR, Lipo-IR, HOMA, AA-IR, and decreased glycine compared to both CT and nonOb_NAFLD (p < 0.004), while BCAA and GNA-AA were higher than in CT but similar in NAFLD (fugure).

Glycine was negatively correlated to Hep-IR (Rho = -0.46; p < 0.0001), Lipo-IR (Rho = -0.39; p = 0.0007) and AA-IR (Rho = -0.28; p = 0.0047). Obesity was independently associated with increased IR and decreased glycine.

Anabolic resistance (AA-IR) correlated with Hep-IR (Rho = 0.89; p < 0.001) and increased with BMI in NAFLD.

Conclusion: Reduced plasma concentrations of glycine and increased concentrations of BCAA are associated with IR in muscle, liver and adipose tissue and anabolic resistance in non-diabetic subjects with NAFLD.

Figure:

	CT	nonOb_NAFLD	Ob_NAFLD
BCAA µmol/L	319.3 ± 11.9	379.3 ± 11.5§	389.6 ± 12.2§
Glutamate µmol/L	121.9 ± 19.2	187.1 ± 12.8*	150.2 ± 15.2#
Glycine µmol/L	206.1 ± 10.2	183.7 ± 5.1**	172.0 ± 5.4**
GNG AA µmol/L	1061.8 ± 45.7	1184.6 ± 22.9*	1165.0 ± 36.4
HOMA (GLU*INS/22.5)	1.7 ± 0.2	2.8 ± 0.3**	3.9 ± 0.4§#
Hep_IR (EGP*INS)	52.1 ± 6.3	105.1 ± 7.8§	161.5 ± 20.0§#
Lipo-IR (Ragly*INS)	4.8 ± 0.8	6.9 ± 0.7**	9.1 ± 1.0§#
AA-IR (BCAA*INS)	2371.8 ± 237.8	4160.1 ± 298.3§	6229.8 ± 660.6§#

Data were presented as Mean ± SEM and compared using Mann–Whitney U test for unpaired samples to determine difference between groups. p<0.005 was considered significant. *p<0.05 vs CT, **p<0.01 vs CT, § p<0.001 vs CT; # p<0.05 vs nonOb_NAFLD, ##p<0.01 vs nonOb _NAFLD, ###p<0.001 vs nonOb _NAFLD.

P01-11YI Increased hepatic Angiopoietin-Like Protein 3 is associated with NAFLD and with liver expression of vitamin D receptor and vitamin D hydroxylases

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Background and aims: Angiopoietin-like (ANGPTL) proteins and vitamin D are differently implicated in the intrahepatic fat accumulation though mechanisms involving either way the vitamin D receptor (VDR) expression on hepatocytes. Hepatic VDR has been demonstrated to promote hepatic steatosis directly and through the modulation of the ANGPTL8-induced intra-hepatocyte triglycerides accumulation. For its function ANGPTL8 requires ANGPTL3, an hepatokine which strongly inhibits circulating lipoprotein lipase (LPL), and whose inactivation reduces hepatic steatosis in experimental models. So far, little is known on ANGPTL3 in human non-alcoholic fatty liver disease (NAFLD) and no data exist on the relationship between vitamin D related genes and ANGPTL3 in NAFLD.

Method: To test the hypothesis of a relationship between ANGPTL3 and VDR expression in NAFLD, we carried out a cross-sectional investigation on forty consecutive obese subjects (mean ± SD age: 43.7 ± 9.6 years) with and without NAFLD, candidate to bariatric surgery and referring to our outpatient clinics at Sapienza University, Rome, Italy. All the participants underwent complete metabolic profiling and intra-operative liver biopsy. Hepatic expression of VDR, ANGPTL3, ANGPTL8, LPL, CYP27A1 and CYP2R1 was evaluated by real-time PCR, VDR expression was further investigated by immunohistochemistry. Serum 25 (OH) vitamin D levels [25 (OH)D] were also measured by colorimetric method (LAISON, DiaSorin).

Results: Patients with biopsy-proven NAFLD/NASH (75%) had significant higher ANGPTL3, VDR and LPL mRNA expression on hepatocytes in comparison with obese individuals with normal liver histology. Liver ANGPTL3 expression tightly correlated with worse NAS steatosis score (0.34, p = 0.035), increased hepatic LPL (0.47, p = 0.002) and with higher expression of VDR (0.34, p = 0.03) and vitamin D hydroxylases, such as CYP27A1 (r = 0.54, p < 0.001) and CYP2R1 (r = 0.45, p = 0.004). No association was found between ANGPTL8 and NAFLD or hepatic VDR expression. Greater VDR mRNA expression was the main determinant of hepatic ANGPTL3 independently of age, sex, presence and severity of NAFLD.

Conclusion: This study demonstrates for the first time the presence of increased hepatic ANGPTL3 levels in condition of NAFLD, likely modulated by the VDR and vitamin D related genes, providing novel insights on mechanisms behind the pathogenesis of NAFLD and opening a new scenario for therapeutic approaches to this condition.

Figure: 0

P01-12 The role endothelial lipase in patients with non-alcoholic fatty liver disease and hypertension depending on the performance of the lipid profile

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) and hypertension are among the most common diseases in the world. One of the negative factors contributing to the formation of cardiovascular risk in patients with NAFLD affected with hypertension is the low level of HDL cholesterol. Since endothelial lipase (EL) is directly related to the metabolism of HDL, we analyzed EL in accordance with the levels of HDL.

Method: 50 patients with NAFLD on the backdrop of hypertension and overweight were examined. Group distribution was performed according to HDL values: group 1 low values (<1.04 mmol/l), n = 10; group 2-moderately reduced (1.04-1.54 mmol/l), n = 27; group 3-protective values (>1.55 mmol/l), n = 13. The patients were comparable by gender and age. The average age was [53 ± 7.5]. The severity of steatosis was determined by the NAFLD index liver fat score. The concentration of EL serum was determined by ELISA using kits of reagents "Aviscera Bioscience INC" (USA).

Results: Analysis of the lipid profile shows a significant difference between all groups in terms of HDL. The concentration of total cholesterol was not significantly different in the groups. At the same time, significantly lower levels of triglycerides and LDL in individuals with high levels of HDL. The results are presented in table 1. Regression analysis demonstrates a significant positive (p = 0, 04) correlation between EL level with total cholesterol and the negative with LDL level.

Conclusion: It turns out that in patients with more pronounced non-alcoholic liver steatosis there is a proatherogenic type dislipidemia with high levels of triglycerides and low levels of protective HDL. The concentration of EL did not show a direct relationship with the level of HDL. However EL was found to depend on LDL and total cholesterol levels, which can determine its complementary role in LDL metabolism in conditions of liver steatosis.

Figure:

Parameters	Group 1 HDL <1, 04 mmol/l n = 10		Group 2 HDL 1, 04-1, 54 mmol/l n = 27		Group 3 HDL >1, 55 mmol/l n = 13		p value <0, 05
	Mean	SD	Mean	SD	Mean	SD	
Total cholesterol, mmol/l	5, 58	1, 02	5, 65	1, 34	5, 87	1, 30	
Triglycerides, mmol/l	2, 24	1, 48	1, 64	0, 80	1, 29	0, 34	13
HDL, mmol/l	0, 91	0, 10	1, 28	0, 16	1, 78	0, 19	12 23 13
LDL, mmol/l	3, 67	0, 75	3, 57	1, 32	3, 41	1, 23	
VLDL, mmol/l	1, 01	0, 67	0, 80	0, 39	0, 58	0, 15	13
Endothelial lipase, ng/ml	11, 39	2, 74	11, 99	4, 49	12, 33	4, 19	13
NAFLD liver fat score	3, 41	4, 29	2, 96	3, 19	0, 43	1, 47	13 23

Tab. 1. Lipid profile and NAFLD liver far score depending on HDL levels.

P01-13YI The genetic background strongly influences the development of steatohepatitis and metabolic syndrome in a novel experimental model of dual ASH/NASH

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Background and aims: Recent clinical studies showed that current Western dietary habits (high fat and high sugar food) and moderate but constant alcohol intake have direct causative link to the epidemic of steatohepatitis and Metabolic syndrome in the prosperous parts of the world. However, it has not been systematically reported in animal models. In the present work, we have investigated strain-dependent differences in the susceptibility to Western diet (WD) and alcohol (*ASH/NASH model*) in three commonly used inbred mouse strains.

Method: C57BL6/J, 129sOla, BALB/c mice received 10% alcohol in the sweetened drinking water together with a WD for 10 weeks (*ASH/NASH model*). Serum markers of liver damage, lipid and glucose metabolism, liver and epididymal white adipose tissue (eWAT) histology, pro-inflammatory and pro-fibrotic markers were analysed.

Results: C57BL6/J animals fed with our novel ASH/NASH diet elicited progressive metabolic perturbations characterized by obesity (increased body weight), eWAT hypertrophy, significant hepatomegaly, basal hyperglycemia and exacerbated glucose intolerance after GTT. Significant liver damage was characterized by elevated plasma AST, profound macrovesicular hepatic steatosis, significant lobular inflammation with intrahepatic accumulation of CD45$^+$-positive immune cells and periportal fibrosis with remarkable collagen accumulation. In sharp contrast, 129sOla and BALB/c animals treated with the novel ASH/NASH diet exhibited significant protection from these detrimental effects. They showed only mild increase of the body weight and no changes in the size of eWAT adipocytes. Importantly, basal levels of glucose and GTT were normal in both strains, as well as plasma transaminases. Remarkably, 129sOla and BALB/c exhibit distinct hepatic phenotype with only minor accumulation of lipids, absence of inflammation and fibrosis in the liver tissue.

Conclusion: Our findings indicate that only C57BL6/J mouse strain develop profound hepatic metabolic defects on ASH/NASH diet, while 129sOla and BALB/c demonstrate strong resistance. Collectively, these differences between strains can have direct impact on research outcome and need to be considered carefully in liver and metabolic studies.

Figure:

P01-14YI Selective modulation of mGlu5 receptor affects oleic and palmitic acid-induced steatosis in HepG2 cells

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Background and aims: Despite extensive research on the pathophysiology of NAFLD, currently, there are still no targeted therapies available. Palmitic and oleic acid are fatty acids (FAs) widely used in *in vitro* to induce steatosis in cultured human liver cell lines thus representing a good model for a rapid analysis of innovative treatments. We previously demonstrated that metabotropic glutamate receptor 5 (mGluR5) is expressed in rat hepatocytes and its blockade leads to hepato-protection from ischemia/reperfusion injury and acetaminophen hepatotoxicity. Aim of this study was to investigate the role of mGluR5 modulation, using the selective agonist dihydroxyphenylglycine (DHPG) and the negative allosteric modulator 2-methyl-6- (phenylethynyl)pyridine (MPEP), in the protection against FA-induced lipid accumulation and lipotoxicity in HepG2 cells.

Method: HepG2 were pretreated for 12 hours with the mGluR5 agonist DHPG100 μM alone or in combination with MPEP 0.3, 3 and 30 μM. Cells were then treated for 12 hours with a 2:1 mixture of oleic and palmitic acid, at 1.5 mM, in a serum free medium supplemented by 1% BSA, in presence of DHPG+MPEP or DHPG alone. Lipid content, cell viability and reactive oxygen species (ROS) production were assessed by Nile Red staining, MTT assay and DCFH-DA staining, respectively. Western Blot analysis of mGluR5 was performed.

Results: mGlu5 receptor was found in HepG2 cells. The incubation with FAs induced a significant increase in lipid intracellular accumulation, when compared with BSA-treated control. The activation of mGluR5 by DHPG increased FA accumulation, when compared with FA-treated cells. The addiction of MPEP 0.3 μM neutralized the DHPG-induced FAs accumulation. No differences were found in cell viability or ROS production among groups.

Conclusion: Our data show for the first time that the mGluR5 in HepG2 cells has a role in lipid intracellular accumulation. In particular, the mGluR5 activation by DHPG increases cell lipid uptake; instead, the blockade by MPEP administration reduces DHPG-induced cell FA accumulation. Moreover, differently from what observed in other models of liver damage, such as ischemia/reperfusion injury and acetaminophen hepatotoxicity, hepato-protection from lipid accumulation, appears to be associated with a ROS-independent mechanism.

P01-15 Liraglutide improves NASH and metabolic disorders in a 3-week diet-induced NASH mouse model

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Background and aims: Non-alcoholic steatohepatitis (NASH) is an emerging health problem worldwide and is associated with an increased risk of both liver and cardiovascular diseases. However, efficacious pharmacological treatment for NASH is lacking. A major issue for preclinical evaluation of potential therapeutics for NASH is the limited number of appropriate animal models, i.e., models that do not require long-term dietary intervention and adequately mimic disease progression in humans. The present study aimed to evaluate a 3-week dietary mouse model of NASH and to validate it by studying the effects of liraglutide, a compound in advanced clinical development for NASH.

Method: C57BL6/J mice were fed a diet high in fat (60%), cholesterol (1.25%) and cholic acid (0.5%) along with 2% hydroxypropyl-β-cyclodextrinin drinking water (HFCC-CDX diet) to promote hepatic cholesterol loading. Histological and biological parameters were measured at 1 and 3 weeks. Following 1-week diet induction, liraglutide was administrated daily for 2 weeks, and then NASH-associated phenotypic aspects were evaluated in comparison with control mice.

Results: Prior to treatment with liraglutide, mice fed the HFCC-CDX diet for 1 week developed liver steatosis and had increased levels of oxidative-stress markers as well as hepatic and systemic inflammation. For mice not treated with liraglutide, these aspects were even more pronounced after 3 weeks of the dietary period, with additional liver insulin resistance and fibrosis. Although treatment with liraglutide did not improve fibrosis, the treatment corrected the diet-induced alterations in glucose metabolism and significantly reduced hepatic steatosis and inflammation.

Conclusion: This study provides a novel 3-week dietary model of mice that rapidly develop NASH features, and this model will be suitable for evaluating the therapeutic efficacy of compounds in preclinical drug development for NASH.

P01-16YI Genes involved in the bile acid transport and metabolism and their contribution to disease predisposition in a cohort of children with biopsy-proven NAFLD

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Background and aims: Bile acids (BA) have been reported to play a role in the liver and energy metabolism. Indeed, BA and genes involved in their metabolism have been shown to be active in the regulation of lipid metabolism, through the interaction of the farnesoid nuclear receptor (FXR); and to modulate the inflammatory response through the activity of the G protein-coupled receptor TGR5. Therefore, it has been hypothesised that BA may be potentially implicated with the mechanism that characterise the occurrence or, more likely, the progression of non-alcoholic fatty liver disease (NAFLD). Thus, some of the proteins of the BA metabolism, including FXR, have been used as targets for treatment to ameliorate steatohepatitis.

Given the importance of the genetic background alongside the environmental component that characterise NAFLD, and the relative paucity of data currently available, we wished to explore the relevance of variants in genes involved in BA homeostasis, transport and metabolism in children with biopsy-proven NAFLD.

Method: One hundred children with NAFLD had DNA sequenced for 25 selected genes involved in bile acid metabolism and transport. Liver biopsies were scored by a hepatohistopathologist according to the Clinical Research Network NAFLD Activity Score. Data were processed using CLC Genomics Workbench by Qiagen.

For analysis all synonymous variants were removed, along with variants in intronic and splicing regions.

Results: Sixty-two boys and 38 girls were included with a median age at biopsy of 13 years (IQR 11, 14) and a median BMI z-score of 1.97 (IQR 1.59, 2.25). Steatosis grade ≥2 was found in 71% of the patients, with a fibrosis stage F≥2 in 65% of the cohort.

A total of 130 nonsynonymous variants were found: 125 missense, 1 loss of function and 5 variants that have not been reported before. The minor allele frequencies (MAF) for the known variants showed no significant differences compared with the MAF of the general population from gnomAD Browser.

The *in silico* effect of each variant on the protein function was also evaluated. Although we obtained conflicting results between Polyphen-2 and SIFT, 53.8% of these variants in Polyphen-2 were considered benign, 30.8% probably damaging and 3.8% were defined as possibly damaging. We assessed the association of these variants with steatosis, NAS and fibrosis stage, with Spearman correlation, but without finding any statistical association.

Conclusion: Though we have not demonstrated statistical significance, variants in genes of the bile acid synthesis and transport were found in those with lesser and greater severity of NAFLD. With further analysis, these findings may help to identify subjects at risk of developing more severe stages of NAFLD and may predict their response to therapies targeting BA homeostasis.

Figure:

Total variants per gene in patients with F≥2 and F<2
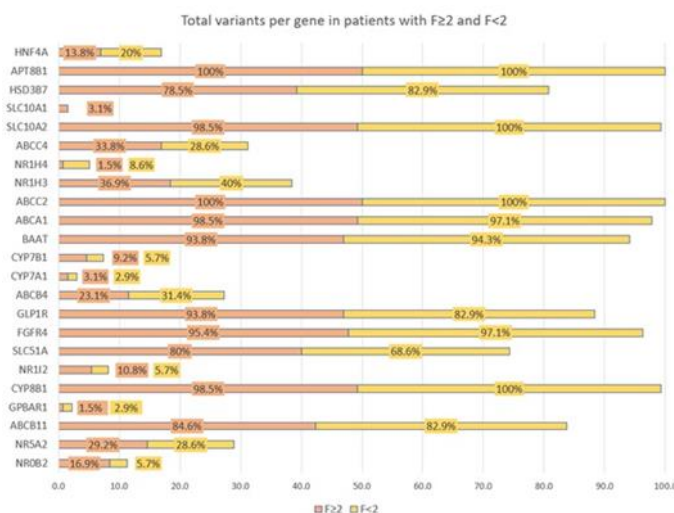
P01-18 In patients with Non-alcoholic Fatty Liver Disease significant fibrosis and the active non-alcoholic steatohepatitis is associated with a moderate-high cardiovascular risk at 10 years

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Background and aims: The presence of non-alcoholic steatohepatitis (NASH) and advanced fibrosis are strongly associated with cardiovascular mortality. Currently it is unknown whether the cut-offs established by non-invasive methods (FIB-4, NAFLD score, and Fibroscan®) or the presence of active histological NASH (NAS≥4) and significant fibrosis (F≥2), could predict cardiovascular risk (CVR). Aims: 1- To evaluate the association between the presence of NASH (NAS≥4-F≥2) in liver biopsy with CVR ≥7.5 at 10 years estimated by atherosclerotic cardiovascular disease (ASCVD). 2- To evaluate the diagnostic capacity of non-invasive methods to estimate an RCV ≥7.5% at 10 years. 3- To evaluate the CVR in patients with NASH lean (BMI <25).

Method: Transversal descriptive study in which patients between 40-75 years without cardiovascular events and histological NASH were included. All the variables were collected at the time of the liver biopsy. To know the factors independently associated with CVR ≥7.5% at 10 years, binary logistic regression analysis was performed. The diagnostic capacity of the non-invasive scores [FIB4 (≥1.3), NAFLD score (≥-1.455) and Fibroscan® (≥8 kPa)] was evaluated by analysis of the area under the curve (AUROC), establishing the respective sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV).

Results: We included 214 patients (55% female), median age of 58 (49-65) years. The prevalence of obesity, smoking, diabetes, hypertension, dyslipidemia and NASH were: 62%, 23%, 41%, 54%, 71% and 8%, respectively. The prevalence of CVR ≥7.5% at 10 years was 54%. The independent factors associated with CVR ≥7.5% at 10 years were the presence of NASH NAS≥4-F≥2 (OR: 2.45, 95% CI 1.27-4.73, p = 0.008), and eGFR MDRD4 (OR: 0.97, 95% CI 0.96-0.98, p <0.001.) The Table summarizes the diagnostic accurency of non-invasive methods and their respective cut-offs to establish a CVR ≥7.5% at 10 years. Patients with LEAN NASH had no significant differences in NASH NAS≥4-F≥2 and CVR at 10 years.

Conclusion: The presence of histological NASH NAS≥4-F≥2 is independently associated with a CVR ≥7.5% at 10 years. The established cut-offs of FIB-4 and NAFLD present a NPV ≈ 70% for RCV ≥7.5% at 10 years. **Table 1:** The diagnostic accurency of non-invasive methods and their respective cut-offs to establish a CVR ≥7.5% at 10 years.

Variable	FIB4 (>1.3)	NAFLD score (1.455)	LSM (≥8 Kpa)
sensitivity	79%	87%	73%
specificity	61%	40%	34%
PPV	70%	63%	56%
NPV	71%	73%	28%
CC	71	65%	55
AUROC OR	0.74	0.72	0.56

P01-19 Efficacy and safety of saroglitazar in management of NAFLD patients using transient elastography: A single center observational study

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Background and aims: Saroglitazar is a novel and potent dual PPAR-α/γ agonist which is approved in India for the management of Diabetic Dyslipidemia and Hypertriglyceridemia. Saroglitazar is undergoing a Phase III clinical trial in India using the "gold standard" paired liver biopsy and Phase II clinical trial in US and Mexico for NAFLD/NASH. Since liver biopsy is not considered feasible in all patients we aimed at exploring the effect of Saroglitazar on Liver Stiffness Measurement (LSM) and Controlled Attenuated Parameter (CAP) using Transient Elastography (FibroScan®)

Method: The sample size planned for the study is 100 patients and treatment follow-up for 1 year. The subjects were included in study on basis of ultrasonography evidence of fatty liver and CAP value>238 dB/m regardless of their LSM value using Echosens FibroScan® 530 compact. Subjects with fatty liver due to other etiology were excluded. The subjects were required to undergo Transient Elastography (FibroScan®) and their LSM and CAP are recorded at baseline, 6 month and 12 month. Saroglitazar 4 mg once daily along with the continuation of drugs for comorbid illnesses was recommended for treatment till 0ne year. Statistical analysis was done using Paired sample student t -test.

Results: This is an interim analysis for 44 patients completing 6 month follow-up, mean age of 48.3 ± 11.9 years; 81.8% males; mean BMI 26.1 ± 4.11 Kg/m^2 and waist circumference 1.02 ± 0.11 m. 43.2 % were diabetic, 45.5 % hypertensive, and 25 % with family history of liver disorder and associated MetS. Mean CAP at baseline was 323 ± 40.5 dB/m which significantly reduced to 297 ± 44.8 dB/m (p <0.001 and 95% CI). There was significant decline in CAP in S3 Stage (n = 32) from 343 ± 26.2 dB/m to 312 ± 38.2 dB/m (p <0.001) at 6 month (Table 1). LSM at baseline 12.8 ± 8.05 kPa was significantly reduced to 10.1 ± 6.41 kPa at 6 month (p < 0.001 at 95% CI). The stagewise analysis of Liver stiffness (LSM) also shown significant improvement in F0-F1, F2 and F4 stage (Table 2). There was one case who had reported mild symptomatic diarrhea.

Conclusion: The interim analysis of this study shows early trends for reduction in steatosis and fibrosis using FibroScan® for patients who were on Saroglitazar. Further studies, including the undergoing Phase III clinical trial would throw further light on the potential role of Saroglitazar.

Table 1: Changes in the CAP values from Baseline as per Steatosis Stage

	S1 Stage (n = 2)	S2 Stage (n = 10)	S3 Stage (n = 32)
Baseline CAP (dB/m)	232 + 2.12	280 + 12.3	343 + 26.2
6 month CAP (dB/m)	214 + 12.02	264 + 31.7	312 + 38.2
p value at 95% CI	0.323	0.206	<0.001

Table 2: Changes in the LSM values from Baseline as per Fibrosis Stage

	Fo-F1 Stage (n = 13)	F2 Stage (n = 13)	F3 Stage (n = 5)	F4 Stage (n = 13)
Baseline LSM (kPa)	6.52 ± 0.741	8.50 ± 0.737	12.5 ± 0.760	23.8 ± 7.25
6 month LSM (kPa)	5.63 ± 1.432	6.43 ± 1.365	12.6 ± 8.514	17 ± 4.75
p value at 95% CI	0.025	<0.001	0.974	<0.001

P01-20YI Utility of applying age-adjusted FIB-4 cutoffs in patients with type 2 diabetes

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Background and aims: Non-invasive scores are widely used to detect/exclude the presence of advanced fibrosis in patients with NAFLD. FIB-4 is a simple score based on age, ALT, AST and platelet count that was developed and validated in patients aged 35-65. A major limitation of FIB-4 is that a significant proportion of patients fall in the indeterminate zone and cannot be correctly classified. The aim of this study is to evaluate whether recently proposed age-adjusted cutoffs can improve its diagnostic performance in patients with T2DM.

Method: We analyzed in a cross-sectional fashion the clinical and metabolic features of a cohort of patients with T2DM who attended our secondary care diabetes clinic between 2013 and 2018. FIB-4 score was calculated as previously described. Patients with other forms of chronic liver disease were excluded from the analysis. Existing FIB-4 thresholds (cutoffs of 1.3 and 2.67 to rule out and rule in advanced fibrosis, respectively) were compared with age-adjusted thresholds (cutoffs of 1.3 in patients <65 and of 2.0 in patients >65 to rule out, and cutoff of 2.67 to rule in advanced fibrosis).

Results: Among the 2770 patients with T2DM in stable clinical conditions who attended our clinic, FIB-4 was obtained in 1375 patients, whose features were not different than those of the entire population (age: 68 ± 12 ys, with 63% older than 65; BMI: 30 ± 6 kg/m2; sex: 59%M, 41%F; T2DM duration: 10 ± 9 ys; HbA1c: 7.4 ± 3.8%). When applying standard thresholds, 715 (52.0%), 561 (40.8%) and 99 (7.2%) of patients fell in the low, intermediate and advanced risk category. This distribution changed to 1039 (77.7%), 200 (15.1%) and 99 (7.2%), respectively when age-adjusted cutoffs were applied.

Conclusion: Recently proposed age-adjusted cutoffs of FIB-4 were applied to the general outpatient T2DM population, where more than half of patients were 65 or older. The use of age-adjusted cutoffs was associated with the exclusion of advanced fibrosis in a higher proportion of patients, thereby reducing the number of indeterminate results and the potential referral to second-line tests.

P01-21 Impact of FAT10 on PPAR-alpha downregulation during NASH progression

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Background and aims: The peroxisome-proliferator-activated receptor alpha (PPAR-alpha) is a nuclear receptor involved in the regulation of lipid metabolism and inflammation in the liver. PPAR-alpha has been identified as a key factor in preventing Non-alcoholic Steatohepatitis (NASH). Unfortunately, PPAR-alpha is downregulated during NASH development in patient's livers. HLA-F adjacent transcription 10 (FAT10), is a protein part of the ubiquitin-like family and is known to be involved in proteasomal degradation, lipid metabolism and inflammation. Thus, FAT10 might play a role in the regulation of PPAR-alpha expression and activity during NASH progression. Therefore, our project aims at understanding the impact of FAT10 on PPAR-alpha expression in hepatocytes *in vivo* in a murine model of NASH and *in vitro* in human hepatocytes.

Method: FAT10 expression was characterized *in vivo* in livers of mice fed five weeks with a choline-deficient high sucrose, high cholesterol (CDAA) diet inducing NASH and correlated to histological grade of NASH. PPAR-alpha gene and protein expression was measured using respectively real time QPCR and histology. The impact of FAT10 overexpression or down-regulation on PPAR-alpha expression was investigated *in vitro* in two human hepatocyte cell lines by using respectively, cytokine treatment, transient transfection or si-RNA FAT10. The expression of FAT10 and PPAR-alpha was studied using real time QPCR and western-blot and lipid droplets were quantified by BODIPY staining. The interaction between FAT10 and PPAR-alpha was assessed with co-immunoprecipitation and protein ligation assay (PLA).

Results: *In vivo* studies show that FAT10 expression is increased in murine NASH livers and is associated to a decreased expression of PPAR-alpha. *In vitro* studies show that FAT10 overexpression reduced the expression of PPAR-alpha while FAT10 down-regulation increased it. Moreover, FAT10 overexpressing cells accumulated more lipid droplets than control cells. Finally, PPAR-alpha and FAT10 co-immunoprecipitated in human hepatocytes, and this interaction was confirmed by PLA.

Conclusion: These observations suggest that FAT10 is overexpressed during diet-induced NASH and correlated with a decrease in PPAR-alpha expression. Furthermore, FAT10 overexpression downregulates the expression of PPAR-alpha leading to an impaired lipid metabolism. This modulation may be due to the direct interaction observed between these two proteins suggesting that FAT10 could be a new modulator of PPAR-alpha in NASH livers.

Figure:
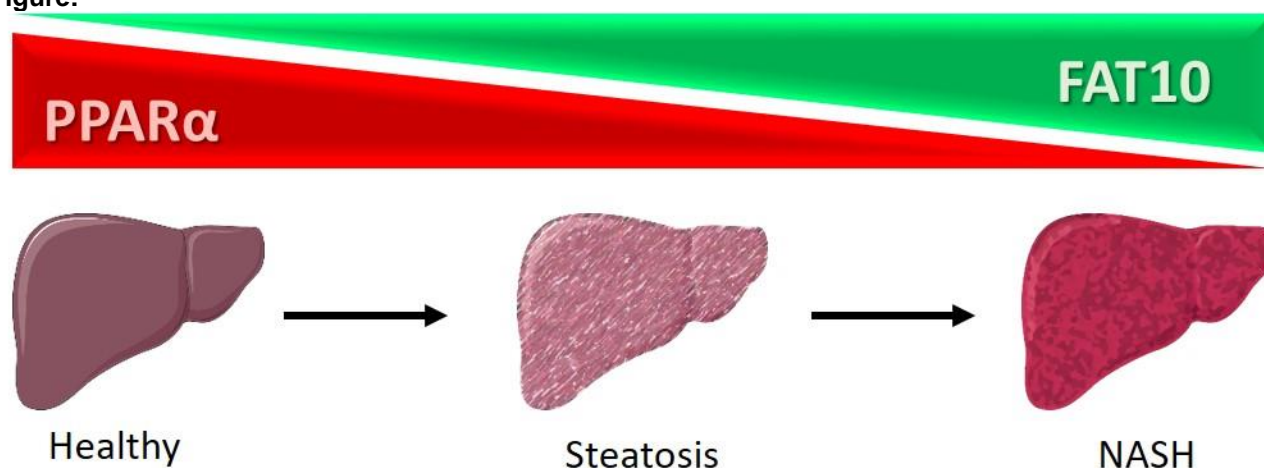
P01-22 Similarity of risk factors among individuals with fatty liver with or without harmful alcohol consumption in a healthy population

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Background and aims: Risk factors for the presence of fatty liver (FL) in a healthy population, have been either the presence of obesity, insulin resistance, and features on the metabolic syndrome, or harmful alcohol consumption. However, there is scarce information regarding the presence of these risk factors in individuals with fatty liver and harmful alcohol consumption. The aim of the present study was to compare these risk factors in an adult population with fatty liver with and without harmful alcohol consumption.

Method: A prospective evaluation of a healthy population, evaluated the presence of hepatic steatosis (ultrasound), as well as anthropometric and biochemical parameters, and the pattern of alcohol consumption, expressed in grams/day. Harmful drinking was considered if >20 g/day in females, and >30 g/day in males. Abstainers and moderated drinkers were considered together as non-harmful drinkers. Insulin resistance (IR) was considered if HOMA test >2.5.

Results: 834 individuals from a population study underwent ultrasound. Those with viral hepatitis markers (HbsAg, or HCV PCR positive) were excluded. FL was present in 297 (36%). From this FL group, 147 were harmful drinkers with fatty liver (EDFL) and 148 were non-harmful drinkers with fatty liver (NEDFL). Age was similar between EDFL and NEFLD (55.2 vs 56.2 years, ns). There was a significant higher prevalence of males in EDLF (M-84.7% vs F-15.3%, p < 0.001), and no gender difference in NEDFL (M-48.3 %vs F-51.7, ns). Obesity was equally prevalent in EDLF and NEFDL (42.9% vs 41.7%, ns). IR was equally prevalent in EDLF and NEFDL (65.4% vs 57.4%, ns). Also, the prevalence of metabolic syndrome (MS) was similar (36.6 vs 40.2%, ns).

Conclusion: Fatty liver associated with harmful alcohol consumption was significantly more frequent in males, while no gender differences were found in the non-harmful drinking group However, the traditional risk factors of NAFLD, such as obesity, MS and IR were similarly present in both groups, thus suggesting that the dichotomy alcoholic and non-alcoholic fatty liver disease is probably artificial and misleading, preventing the focus on correction of metabolic risk factors in individuals with harmful alcohol consumption.

P02-01YI Berberis Aristata, Elaeis Guineensis and Coffea Canephora extracts modulate the insulin receptor expression and improve hepatic steatosis in NAFLD patients: a pilot study

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is common worldwide and is closely associated with insulin resistance and type 2 diabetes. A reduction in insulin receptor (IR) expression has been reported in patients with insulin resistance and diabetes. The aims of this study were to evaluate the effects of a mixture of plant extracts consisting of Berberis Aristata, Elaeis Guineensis and Coffea Canephora (Trixy®, Nathura S.p.A., Italy) on the improvement of glycaemic profile, through the modulation of insulin receptor (IR) levels, and of hepatic steatosis, evaluated with Controlled Attenuation Parameter (CAP) values, in NAFLD patients.

Method: Forty-nine patients (M/F 33/16) with NAFLD and grade of steatosis S1-S2 (CAP value between 214-311 dB/m) were randomly allocated to the Plant Extracts (n = 26) or placebo (n = 23) group (1 tablet per day for six months) without difference in the proportion of the gender between the groups. The median age was 51.5 ± 10.9 years and 57 ± 12.1 years in Plant Extracts and placebo group, respectively. Hepatic steatosis was evaluated, at baseline and at the end of the study, using transient elastography with CAP. Glucose and insulin levels were measured in serum samples. The IR levels were analyzed, in serum samples, by ELISA assay.

Results: At the end of study, in the group treated with plant extracts, patients displayed a significant reduction of serum glucose ($p < 0.001$), insulin levels ($p < 0.01$) and HOMA-IR index ($p < 0.001$) compared to placebo group. Moreover, the IR expression was increased, significantly, in plant extracts group compared to placebo group ($p < 0.05$). Furthermore, there was a significant improvement of hepatic steatosis with a reduction of CAP values in treated group (251.3 ± 41.5 dB/m) compared to placebo group (281.7 ± 35.3) ($p < 0.01$).

Conclusion: The combination of Berberis Aristata, Elaeis Guineensis and Coffea Canephora improves hepatic steatosis and increases serum IR levels, determining a significant amelioration of glycaemic profile in NAFLD patients.

P02-02 The economic burden of patients diagnosed with non-alcoholic steatohepatitis in France, Germany, Italy, Spain and the United Kingdom in 2018

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Background and aims: Non-alcoholic steatohepatitis (NASH) is a chronic disease that can progress to end-stage liver disease. The risk of progression is higher and particularly rapid in people with advanced liver fibrosis due to NASH (stages F3-F4). Early-stage (F0-F2) NASH usually has minimal symptomatology, with a large proportion of the prevalent population likely to be undiagnosed and therefore not receiving interventions to manage their condition. This study estimated the disease burden and economic impact of NASH in adult patients in the European Union 5 (EU5) countries.

Method: A cost-of-illness methodology applying a prevalence approach and World Health Organisation societal well-being measures was used to estimate the prevalence and socioeconomic burden of NASH (F0-F4, hepatocellular carcinoma and liver transplant) in 2018. The analysis and resource-use were based on extensive literature review and consultation with clinical experts, health economists and patient groups, reflecting current clinical practice. Epidemiological estimates were derived from two modelling studies (upper and lower bound). Costs were sourced from literature and national or local fee schedules.

Results: It was estimated that in the EU5 in 2018, 4.0-8.5 million adults were living with F0-F2 NASH, and 0.9-2.0 million adults were living with F3-F4 NASH. Of these, only 4.8-5.5 % of patients with F0-F2 NASH, and 37.8-39.1 % with F3-F4 NASH, were diagnosed and under medical care. The proportion of patients with advanced fibrosis due to NASH who had received a diagnosis therefore comprised between 0.15 % and 0.33 % of the total EU5 adult population in 2018. Average health system costs were € 1, 244 to € 1, 470 per person with any-stage NASH, and € 2, 875 per person with F3-F4 NASH. Overall, total economic costs for all-stage NASH ranged from € 6, 065 to € 13, 424 million, and direct health system costs from € 619 to € 1, 292 million. Total well-being costs ranged from € 41, 536 to € 90, 379 million, primarily driven by the high mortality rate of NASH patients.

Conclusion: This study identified a low level of diagnosis of advanced liver fibrosis due to NASH (F3-F4) in the EU5 countries in 2018. However, there were notable variations in the prevalence of all-stage NASH and the associated direct economic and well-being costs. Total health system costs were greater in patients with advanced fibrosis due to NASH (F3-F4).

P02-03 Cardiovascular risk assessment in a non-alcoholic fatty liver disease group of romanian patients

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Background and aims: The overall risk resulting from traditional risk factors along with intra-abdominal obesity is known as **global cardiometabolic risk**.

Method: We aimed to evaluate the cardiovascular risk of patients with non-alcoholic fatty liver.
We calculated cardiovascular risk in 125 non-alcoholic fatty liver disease patients- group A and 34 subjects in the control group using two forms of clinical practice assessment-Framingham score and SCORE-HeartScore ® formula. We also evaluated patients in terms of two new combinations of clinical factors and laboratory predictors of cardiovascular risk : hypertriglyceridemic waist and hypertensive waist.

Results: Hypertriglyceridemic waist is defined as the simultaneous presence of abdominal circumference above the normal range associated with serum triglycerides above 150 mg%. In patients of group A the hypertrigliceridemic waist prevalence was 43.2% in men and 46.6% in women, with a total prevalence of 45.5%, unlike the control group, where this condition was present for only 2 women.
Hypertensive waist is defined as simultaneous presence of abdominal circumference above the normal range associated with systemic hypertension (Systolic Blood Pressure>140mmHg or antihypertensive treatment). In patients of group A the hypertrigliceridemic waist prevalence was 43.2% in men (n = 16) and 46.6% in women (n = 41), with a total prevalence of 45.5% (n = 57), unlike the control group, where this condition was present in 2 women (5.8%) and in no man.
We calculated the Framingham score risk in patients aged 30-74 years in the 2 groups and got the next Results: a total of 113 patients in group A were framed in terms of age with an average risk of 12.21239%. The risk in the control group was 3.473684%, calculated for the 19 subjects who fell under the criteria of age.
In patients of group A which were included in the age criteria for calculating cardiovascular HeartScore ® risk (n = 101), we obtained an average of **3.03%** statistically significant higher risk than the people the same age and sex (1.88 %).
A total of 20 patients in group A showed an increased cardiovascular risk (≥5) quantify into the SCORE system, mostly men.
Estimation of Framingham and SCORE cardiovascular risk proved an increased risk with age (Spearman coefficient r = 0.64, respectively r = 0.47).

Conclusion: The risk was lower in female sex and higher in those presenting obesity, hypertensive waist or metabolic syndrome. The clinical diagnosis of metabolic syndrome is not sufficient to assess the risk of cardiovascular disease. In order to appropriate assessment and management of overall cardiovascular risk in clinical practice, is important to take into account the traditional risk factors and the additional contribution brought by obesity/insulin resistance and their related complications.

P02-04YI Is diabetes mellitus associated with hepatocellular carcinoma in patients with chronic liver disease of non viral etiology?: A case control study

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Background and aims: Hepatitis B, hepatitis C, alcohol, and non-alcoholic steatohepatitis are important etiological factors for hepatocellular carcinoma (HCC). Role of diabetes mellitus (DM) as a contributory factor for HCC in patients with viral etiology has been adequately demonstrated, however its role in HCC due to alcohol and NASH remains controversial. This case-control study aimed to investigate the association of DM with HCC in patients with alcoholic liver disease and cryptogenic (including NASH-related) liver disease

Method: We conducted this case-control study at Sir Ganga Ram Hospital, Delhi, India. Consecutive patients of HCC due to alcohol or cryptogenic etiologies presenting between 2012 and 2018 were included in the study as cases. Age and sex matched patients of chronic liver disease of same etiologies, presenting during the same period, were chosen as controls. Cases and controls were in the ratio of 1:2. Patients of any other etiologies were excluded. Prevalence of DM among cases and controls were compared

Results: A total of 138 patients of HCC (mean age 61 ± 9 years, 95% males) were included in the study. The etiologies of HCC were cryptogenic (including NASH) 54%, and alcohol 46%. DM was present in 48% of patients. A total of 276 controls (mean age 61 ± 7 years, 92% males; p = NS compared to cases) were included in the study. Among patients of HCC due to cryptogenic/NASH etiology, the prevalence of DM was significantly higher than in controls (p = 0.012; OR 2.3, 95% CI 1.2, 4.3). Among patients of HCC due to alcohol etiology the prevalence of DM was similar to that of controls (p = NS).

Conclusion: DM is strongly associated with the increased risk of HCC in patients of cryptogenic/NASH etiology. Therefore, these patients represent a high HCC risk population and should be considered for closer HCC surveillance program. DM does not seem to increase the risk of HCC in patients of chronic liver disease due to alcohol etiology

Figure:
Table1

Cryptogenic/NASH Etiology					
	HCC (n = 75)	Controls (n = 86)	P value	Odds ratio	95% C.I.
Diabetes present	45 (60%)	34 (40%)	0.012	2.3	1.2, 4.3
Diabetes absent	30 (40%)	52 (60%)			

Alcohol Etiology					
	HCC (n = 63)	Controls (n = 190)	P value	Odds ratio	95% C.I.
Diabetes present	21 (33%)	45 (24%)			
Diabetes absent	42 (67%)	145 (76%)	0.139	1.6	0.9, 3.0

	Hcv	hbv	ethanol	Nash
347	87	98	67	37
	25	28	19	11
298	25	29	14	17+15

HCC

- MIXED & OTHERS 15%
- HCV 25%
- NASH 17%
- HBV 29%
- ETHANOL 14%
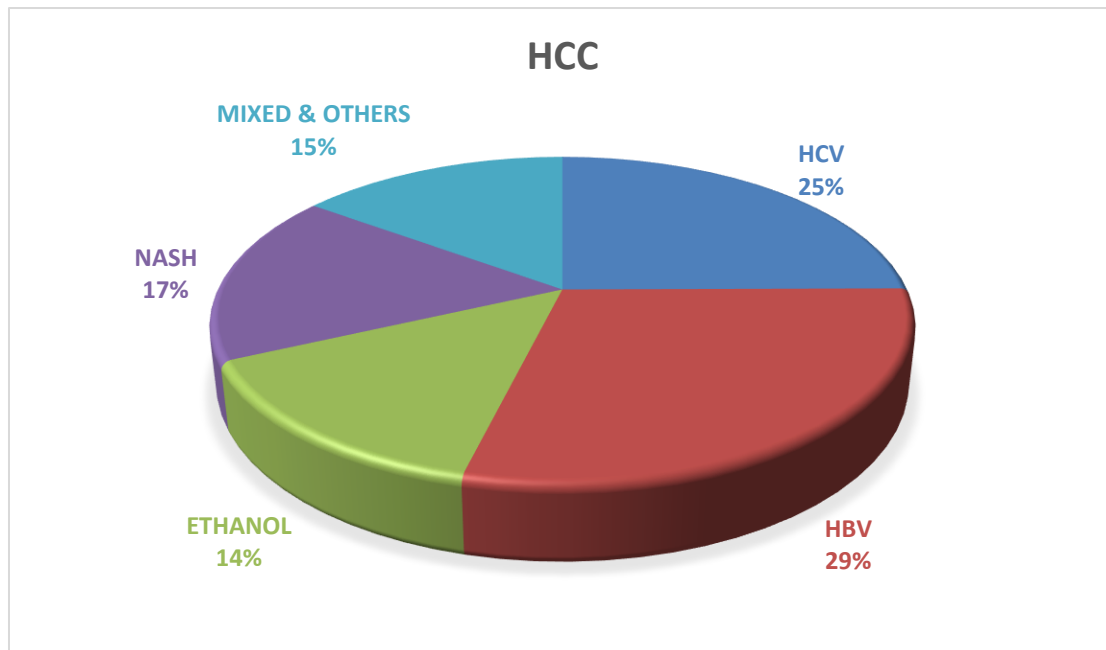
P02-05YI Picroside II protects FFA-induced lipid accumulation and lipotoxicity in an in-vitro model of NAFLD

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Background: Accumulation of free fatty acids (FFAs) within the hepatocytes is the hallmark of NAFLD. Excessive deposition of FFAs alters lipid metabolism pathways increasing oxidative stress and mitochondrial dysfunction. Attenuating hepatic lipid accumulation, oxidative stress and improving mitochondrial function could provide potential targets in preventing progression of NAFL to NASH. Earlier studies with extract *Picrorhiza kurroa (Pk)* have shown reduction in hepatic damage and fatty acid infiltration in several experimental models and clinically in viral hepatitis. The effect of *Pk's* phytoactive, picroside II (PII) thus needed mechanistic investigation in appropriate *in vitro* liver cell models.
Aim: To study the effects of PII on FFAs accumulation, oxidative stress and mitochondrial function *vis-a-vis* silibinin (SIL), a positive control in an *in vitro* NAFLD model.
Methods and Results: HepG2 cells were incubated with FFAs-500 µM and 1000 µM for 20 hours. Picroside II-10 µM inhibited FFAs-induced lipid accumulation via attenuating the expression of FATP5, SREBP1c, SCD1, FOXO1 and PEPCK. PII also prevented FFA -induced loss of the mitochondrial membrane potential ($\Delta\Psi m$), ATP depletion and production of reactive oxygen species (ROS). The gain in $\Delta\Psi m$ and ATP production is indicative of increase in expression of Cytochrome C-mRNA and protein. Increase in the expression of MnSOD, catalase and higher levels of tGSH and GSH:GSSG ratio explained the ROS salvaging of PII. SIL showed parallel activities in some targets.
Conclusion: The findings suggest that PII attenuates hepatic lipid accumulation by decrease in FFAs uptake, lipogenesis and gluconeogenesis. PII significantly attenuated FFAs-induced lipotoxicity. The reduction in ROS, increase in antioxidant enzymes and improvement in mitochondrial function underlie the mechanisms of action of PII. This suggests the need to develop an investigational drug profile of PII for NAFLD as a therapeutic strategy. This could be evaluated through the fast track path of Reverse Pharmacology.
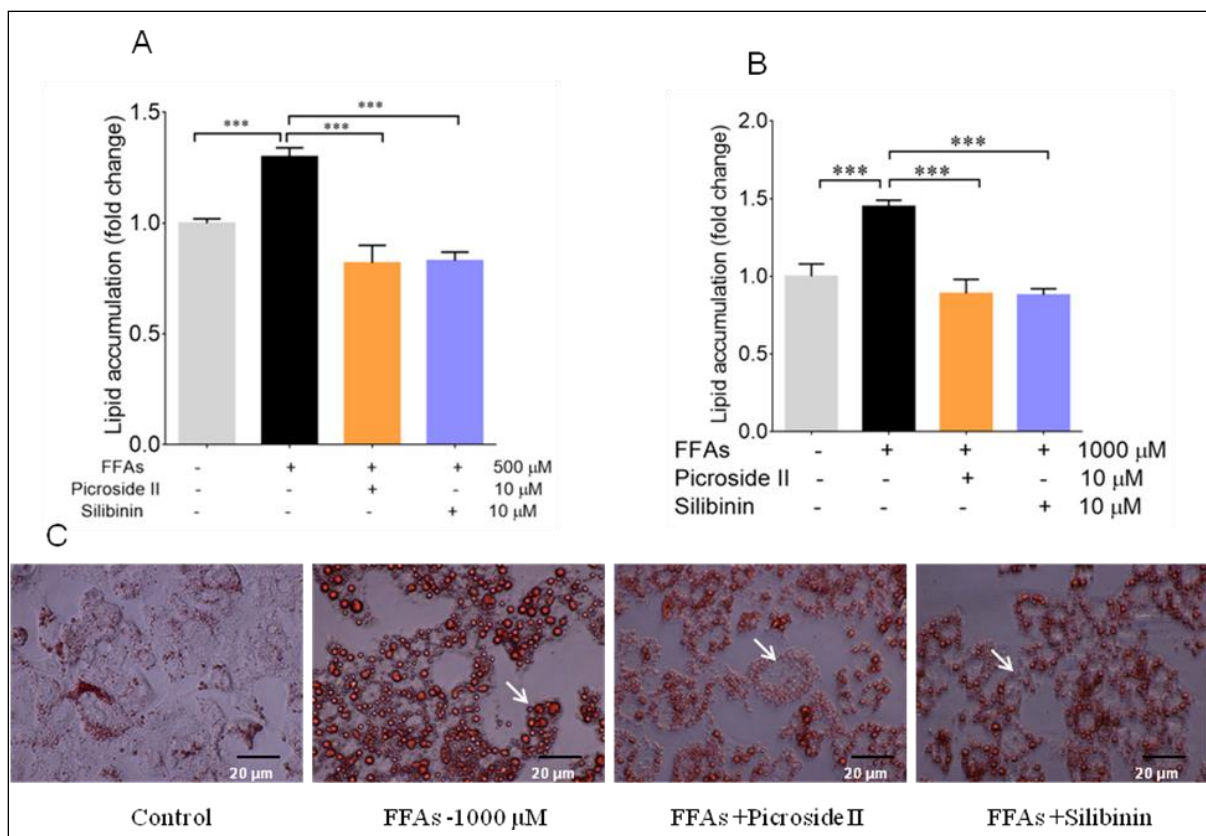
Figure 1: PII attenuates FFAs-mediated lipid accumulation: HepG2 cells were pretreated with PII and SIL at a concentration of 10 uM for 2 hours, followed by FFAs (500 uM and 1000 µM) challenge for another 20 hours. ORO colorimetric assay (A and B) and ORO staining (10X) (C). The values are expressed as mean ± SEM from six independent experimental repeats. ***$p < 0.001$.

P02-06YI Liver damage in non-alcoholic fatty liver disease: Changes in stearoyl-CoA-desaturase index and metalloproteinase activity

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Background and aims: Stearoyl-CoA-desaturase-1 (SCD-1) is the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids (MUFAs) from saturated fatty acids (SFA). SCD-1-deficiency attenuates the induction of tumor necrosis factor (TNF)-alpha (Liu et al., 2010). The progression of NAFLD to NASH in obesity is modulated by TNF-alpha (Ezquerro et al., 2018) and characterized by extracellular matrix remodelling (gelatinase activity) (Munsterman et al., 2018). Aim of this study was to investigate the modifications of SCD-1 index and gelatinase activity (MMP-2 and MMP-9) in fatty livers obtained from two rat models of NAFLD, normal rats fed methionine and choline deficient (MCD) diet versus genetically Obese Zucker rats.

Method: Eight-week-old male Wistar rats (n = 14) fed for 3-week with MCD diet and relative control diet were used as nutritional model of NAFLD. In parallel, twelve-week-old Obese and Lean male Zucker rats (n = 14) were used as genetic model of NAFLD. Serum levels of hepatic enzymes (AST, ALT, Alkaline Phosphatase) and TNF-alpha were quantified. Hepatic metalloproteinases, MMP-2 and MMP-9, were evaluated by zymography. Liver fatty acid profiling was performed by Gas Chromatography-Mass Spectrometry analysis (GC-MS). SCD-1 index as ratios of SCD product and precursor fatty acids (16:1n−7/16:0 as D9-16D and 18:1n−9/18:0 as D9-18D) have been investigated.

Results: Liver D9-18D index grew both in MCD and Obese Zucker rats with a three-fold increase in MCD rats. Higher D9-16D index occurred in Obese Zucker rats when compared with MCD group. MMP-2 activity was higher in MCD versus Obese Zucker rats while MMP-9 activity was increased in MCD group and not detectable in Obese Zucker rats. A significant positive correlation was found in MCD group comparing liver levels of D9-18D versus MMP-2 and MMP-9 (p = 0.03 and P = 0.05, respectively). A negative correlation between D9-18D versus MMP-2 was found in Obese Zucker rats (p = 0.01). TNF-alpha increased only in MCD rats and correlated with D9-18D (P<0.001). No significant difference in serum AST, ALT and Alkaline Phosphatase were found comparing the two NAFLD animal models.

Conclusion: MCD rats, that spontaneously progress to NASH, exhibit an increased SCD-1 index correlating with MMP activation and TNF-alpha levels. Our data support the emerging evidences indicating the role of SCD-1 in fatty liver inflammation and the involvement of metalloproteinase in the development of liver injury.

P02-07 Effect of obeticholic acid on liver function in patients with fibrosis due to NASH

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Background and aims: Obeticholic acid (OCA), a potent and selective FXR agonist, has been shown to improve fibrosis in NASH patients in a pivotal Phase 3 study (REGENERATE). As part of the OCA development program, a study in patients (pts) with NASH evaluated the effect of OCA on liver function improvement using the HepQuant methodology. HepQuant measures the hepatic extraction of exogenously administered labeled cholate as a marker of liver function, which has been correlated to clinical outcomes using a Disease Severity Index (DSI). Based on prior studies of the relationship of DSI to probability of varices, a 2-point decrease in DSI is considered clinically meaningful. The aim of this analysis was to measure liver function in NASH patients with fibrosis after 3 months of OCA treatment.
Method: 51 pts were randomized 1:2:2 to placebo, OCA 10 mg, or OCA 25 mg QD for 85 days. Labeled cholate was administered intravenously and orally on Day -1 (baseline), 8, and 85 for HepQuant assessment.
Results: 50 pts, primarily white with median age 55yrs and BMI 35kg/m^2 completed Day 85. 45 pts had a DSI at baseline (27% F1, 62% F2/3, 11% F4); 43 pts had both a baseline and Day 85 DSI assessment. The mean baseline DSI ± SD (n) was for F1 16.4 ± 3.8 (n = 12), F2/3 19.0 ± 4.6 (n = 28), and F4 22.1 ± 6.7 (n = 5). The mean baseline DSI score for all pts (F1-F4) was consistent with an increased likelihood for varices based on previous results in NASH and HCV pts[1]. OCA treatment improved hepatic function as evidenced by the number of responders (>2-point decrease) and median decrease in DSI at Day 85 (Table). No unexpected safety findings were observed.
Conclusion: This is the first demonstration of OCA eliciting a dose-dependent clinically significant improvement in liver function in NASH. These results are consistent with the dose-dependent reversal of fibrosis observed in REGENERATE and further support the efficacy of OCA treatment in pts with fibrosis due to NASH.

Figure:

	Placebo	OCA 10 mg	OCA 25 mg
F2/F3			
Responders	0% (0/5)	36% (4/11)	73% (8/11)
ΔDSI (Median)	-0.65	0.78	-3.81
All (F1-F4)			
Responders	10% (1/10)	44% (7/16)	59% (10/17)
ΔDSI (Median)	-1.07	-1.17	-2.78

P02-08YI Differential effects of palmitic acid on the development of NASH and related metabolic disorders

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Background and aims: The free fatty acid (FFA) composition of the diet can dramatically alter metabolic pathways. Particularly, a diet rich in saturated FFA (i.e. Palmitic Acid, PA) not only can significantly impact on lipid and glucose metabolism, but also determine the susceptibility to metabolic abnormalities and liver injury.
The aim of this study was i) to test the hypothesis that both quantitative and qualitative aspects of the dietary fat exert a pivotal impact on the development of metabolic disorders, and; ii) to determine the effects of a well-known fatty acid, PA, in NASH, obesity and glucose intolerance development.
Method: In our study we used two types of Western diet (WD)with similar content of total fat but different concentration of PA, high (HPA-WD) (3, 3%) and low (LPA-WD) (1, 51%). C57BL/6 male mice were fed with HPA-WD (n = 7), LPA-WD (n = 7) and a normal chow diet (control group, n = 7) for 13 weeks. Upon sacrifice, body weight (BW), liver weight (LW), hepatic tissue and epididymal fat (eWAT) were thoroughly analyzed. In addition, glucose tolerance test (GTT) and serum markers of liver injury were assessed.
Results: Feeding with either a HPA- or a LPA-WD caused significant weight gain and moderate obesity at the end of the study. However, the HPA-WD triggered much more pronounced effects characterized by prominent hepatomegaly and increase in the eWAT/BW ratio. Moreover, mice fed with HPA-WD exhibited signs of liver injury associated with elevated serum transaminases (AST and ALT) and increased LDH activity. Notably the HPA-WD diet also resulted in increased hepatic fibrosis and remarkable collagen accumulation in periportal areas. Interestingly, glucose tolerance after glucose load was impaired only in the HPA-WD fed group. Furthermore, HPA-WD fed mice developed profound eWAT inflammation characterized by macrophage infiltration and a dramatic increase in crown-like structures.
Conclusion: Our results demonstrate that a diet containing a high concentration of PA promotes liver injury, hepatic fibrosis induces adipose tissue expansion and inflammation, and exacerbates glucose intolerance. Altogether, these data provide important basis for further clinical studies on the dietary pattern of patients with NASH and related metabolic disorders.

P02-09YI Deletion of Keap1 and L-selectin in mice protects from NAFLD progression

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Background and aims: Lipotoxicity-driven oxidative damage, aberrant immune cell infiltration and dysregulated fibrosis have been implicated in the "multiple hits" driving NAFLD progression. The development of treatment strategies needs a better understanding of these processes and their interactions. Previously we have shown that L-selectin-deficient mice are protected from experimentally induced NASH. Amelioration of disease is accompanied by decreased infiltration of neutrophils into the liver and a reduced oxidative stress response. In the present study we aimed to analyze the outcome of a migration blockade caused by selectin deficiency in combination with the inhibition of a second hit, namely oxidative damage, caused by hepatocyte-specific Kelch-like ECH-associated protein1 (Keap1) deletion. Inhibition of Keap1 activates the transcription factor nuclear factor erythroid 2-related factor (Nrf)-2 thereby causing the expression of genes that mediate anti-oxidative stress responses.

Method: L-selectin-deficient mice with hepatocyte-specific Keap1 deletion (albumin- (Alb)-Cre$^+$L-sel$^{-/-}$/Keap1$^{\Delta hepa}$) and L-selectin-deficient littermates (L-sel$^{-/-}$/Keap1$^{flx/flx}$) were subjected to 24 weeks of western-style diet (WD). Experimentally induced metabolic and liver changes were evaluated in terms of symptomatic and histologic parameters, flow cytometry analysis, and expression analysis of inflammatory mediators.

Results: L-sel$^{-/-}$/Keap1$^{\Delta hepa}$ mice exhibited a reduced disease severity as demonstrated by lowered weight gain and adiposity, a significantly improved ability to tolerate glucose, and lowered values of serum ALT in comparison to L-sel$^{-/-}$/Keap1$^{flx/flx}$ mice. Flow cytometric analysis showed no differences in absolute cell numbers of innate or adaptive immune cell composition of the liver, but a reduced influx of CD11b$^+$ F4/80$^+$ inflammatory monocyte-macrophages into the epididymal adipose tissue of L-sel$^{-/-}$/Keap1$^{\Delta hepa}$ mice was observed. In addition, the fat tissue of the L-sel$^{-/-}$/Keap1$^{flx/flx}$ mice contained a significantly higher number of cytotoxic CD8$^+$ T cells, typically associated with a pro-inflammatory milieu.

Conclusion: The combined deletion of hepatocytic Keap1 and L-selectin resulted in improved metabolic syndrome, reduced liver damage and inflammation meaning that simultaneous targeting of adhesion-molecule mediated immune cell migration and upregulation of anti-oxidative mechanisms could give insights into the underlying pathogenesis of NAFLD.

P02-10YI The relationship of systemic inflammation and endothelial dysfunction with blood lipid spectrum and adipokines levels in patients with NAFLD

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Background and aims: The unbalanced production of adiponectin and TNF-α contributes to the damage of many tissues, including the liver. Adiponectin counteracts the synthesis of TNF-α, which in turn inhibits the activity of adiponectin.
The aim-establish a relationship between proinflammatory cytokines and adipokines with endothelial dysfunction and blood lipid spectrum in patients with NAFLD.
Method: The study involved 176 patients with NAFLD with normal, overweight and obese, and 68 patients without NAFLD. We determined the level of inflammatory mediator, endothelin (ET-1), the thickness of the intima-media complex, presence atherosclerotic plaque and stenosis of the carotid arteries. Conducted anthropometric survey, measured the degree of liver fibrosis using elastography.
Results: We found a violation of lipid and carbohydrate metabolism, increased proinflammatory cytokines and decreased adiponectin in patients with liver steatosis. Levels of adiponectin in serum were related with levels of ET-1, HDL-cholesterol, TNF- α and GGT, while the level of TNF- α was related with, level of ET-1, insulin resistance, level of adiponectin and liver steatosis. The inverse correlation between the levels of adiponectin and liver steatosis degree, total cholesterol, TNF-α levels and index HOMA-IR was found. The levels of TNF-α had a strong direct correlation with steatosis, levels of ET-1 and index HOMA- IR.
Conclusion: Cytokine imbalance and reducing of adiponectin levels contributes to development of endothelial dysfunction increasing total cholesterol level and accumulation of fat in the liver.
Figure:
Relationship of the concentration of C-reactive protein with the indicator of the decimal logarithm of the ratio between the content of adiponectin and leptin in NAFLD patients with overweight and obesity.
Note: r-Spearman correlation coefficient; p-the reliability of the correlation coefficient.

Scatterplot of **CRP** against **Ig (A/L)**

CRP = 18,7052-4,4763*x

Ig (A/L):CRP: r = -0,5468; p = 0.0000
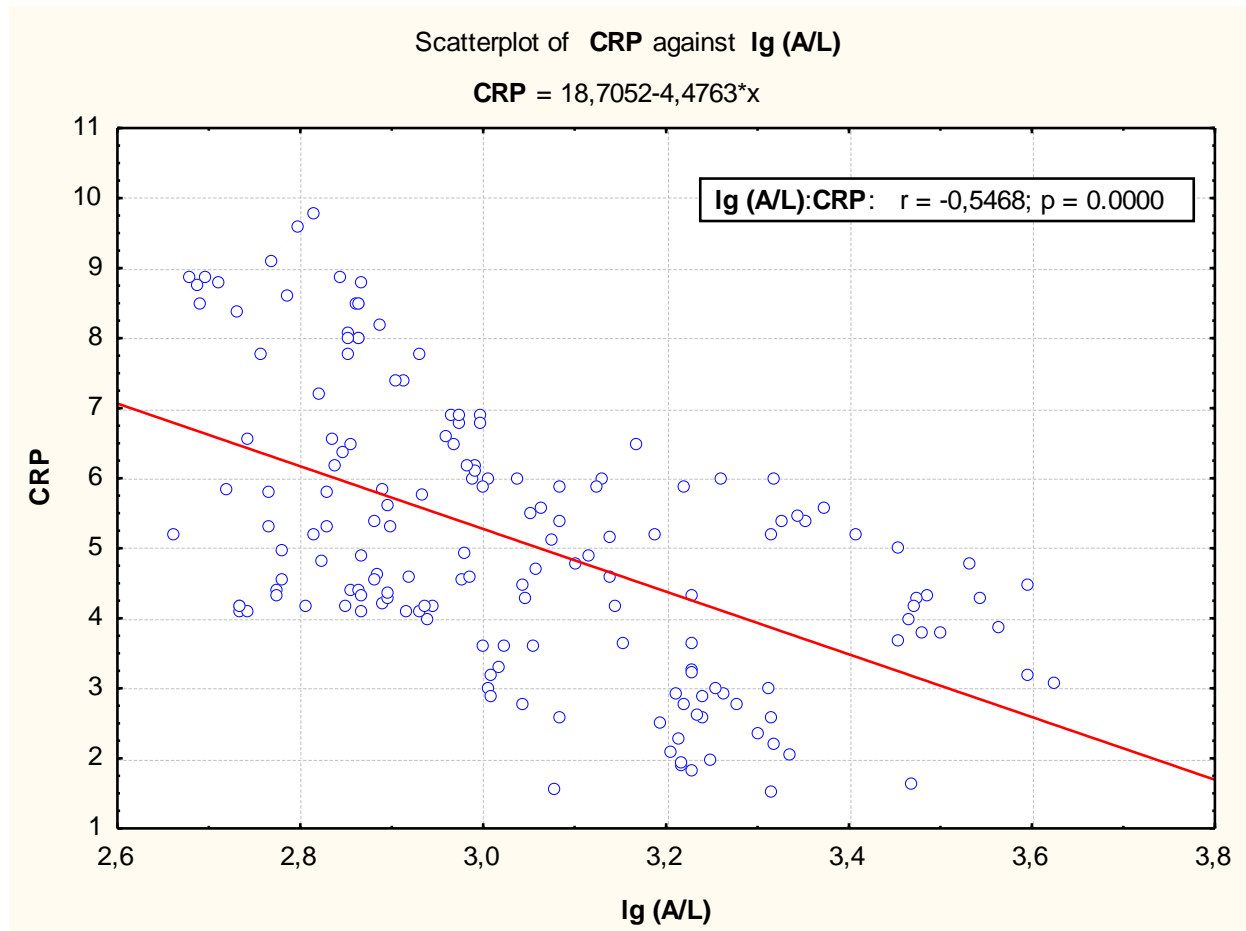
P02-11 Effect of elafibranor treatment and dietary intervention in the Gubra Amylin NASH (GAN) diet-induced obese mouse model of biopsy-confirmed non-alcoholic steatohepatitis

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Background and aims: The trans-fat containing AMLN diet has been extensively validated in C57BL/6J mice for reliably inducing metabolic and hepatopathological changes recapitulating hallmarks of non-alcoholic steatohepatitis (NASH). Due to a recent ban on trans-fats, we have recently introduced a trans-fat-free diet high in palm oil (Gubra Amylin NASH (GAN)) with similar disease-inducing properties. Here, we characterized the therapeutic effects of elafibranor (PPAR-α/δ agonist) and dietary intervention in GAN diet-induced obese (DIO) NASH mice.

Method: Male C57BL/6J mice were fed GAN diet (40 kcal-% fat, 22% fructose, 10% sucrose, 2% cholesterol; D09100310, Research Diets) for 28 weeks prior to liver biopsy procedure. Only animals with biopsy-confirmed steatosis (score ≥2) and fibrosis (stage ≥F1) were included and stratified into treatment groups. GAN DIO-NASH mice received vehicle (PO, QD), elafibranor (30 mg/kg, PO, QD), or vehicle plus dietary intervention by shifting to chow feeding, for 8 weeks. Vehicle-dosed chow-fed C57BL/6J mice served as normal controls. Pre-post liver biopsy histology was performed for within-subject evaluation of NAFLD Activity Score (NAS) and fibrosis stage. Terminal quantitative liver histology, liver whole-transcriptome analysis, blood and liver biochemistry were assessed.

Results: Elafibranor and dietary intervention induced a weight loss of 18% and 14%. While dietary intervention reduced hepatomegaly, elafibranor treatment increased liver weight. Both treatments improved blood (total cholesterol, ALT, AST) and liver (total triglycerides and cholesterol) biochemistry. Elafibranor and dietary intervention promoted similar improvements in composite NAS without reducing fibrosis stage. Histopathological scores were supported by significant reductions in quantitative levels (% fractional area) of liver lipid, galectin-3 and α-SMA without changes in Col1a1 deposition. Elafibranor treatment and dietary intervention, however, both led to consistently lowered expression of hepatic genes associated with inflammation and fibrogenesis.

Conclusion: Pharmacological and dietary intervention improved metabolic, biochemical and histopathological parameters in GAN DIO-NASH mice with biopsy-confirmed liver pathology including fibrosis. The lack of improved fibrosis histology in the context of notable reductions in pro-fibrotic gene expression following elafibranor administration and dietary intervention suggests that prolonged treatment periods could lead to anti-fibrotic effects.

P02-12 Mitochondrial GNMT-complex II interaction is recovered by miR-873-5p targeting in NAFLD

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Background and aims: Non-alcoholic fatty liver disease is one of the major chronic liver diseases in developed countries, including alterations from steatosis and non-alcoholic steatohepatitis to advanced fibrosis in at risk patients. Non-alcoholic fatty liver disease patients with and without fibrosis may develop hepatocellular carcinoma. Non-alcoholic fatty liver disease -related mechanisms include impairments in lipid uptake, mitochondrial fatty acid beta-oxidation, de novo lipogenesis and/or inefficient very-low-density lipoprotein assembly and secretion.
Glycine-N-methyltransferase (GNMT) is the enzyme responsible for a large amount of transmethylation reactions, comprising 1% of the soluble protein in liver. The importance of GNMT is to maintain the SAMe/SAH ratio, which represents an indicator of the methylation capacity of the cell. In this work we explore the involvement of GNMT in non-alcoholic fatty liver disease pathogenesis and the influence of the GNMT regulator miR-873-5p targeting in fatty liver progression.
Method: We evaluate the miR-873-5p as biomarker in serum samples and liver biopsies of non-alcoholic
fatty liver disease patients. In addition, we employ *in vitro* and *in vivo* non-alcoholic fatty liver disease models to assess the role of GNMT in fatty liver progression, and the targeting of the miRNA miR-873-5p as non-alcoholic fatty liver disease therapy.
Results: In the present study, we describe for the first time the role of GNMT in the mitochondria, particularly interacting with the complex II in the electron transport chain, and increasing mitochondrial functionality and fatty acid beta-oxidation. There is also a decrease in hepatic oxidative stress, protecting it from fatty liver progression.
In particular, miR-873-5p was found to be upregulated in liver and serum from non-alcoholic fatty liver disease patients, correlating with GNMT depletion. Treatment of diet-induced non-alcoholic fatty liver disease in mice based on anti-miR-873-5p improved GNMT mitochondrial function.
Conclusion: In conclusion, we show the potential of miR-873-5p as novel non-alcoholic fatty liver disease biomarker and introduce a new therapy by targeting this microRNA, which results in the restoration of GNMT levels and the improvement of the newly identified GNMT function in mitochondria.

P02-13YI Impact of BMI and Ethnicity on histology as assessed by automated quantitation in liver biopsies of patients with NAFLD

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__Background and aims:__ Histology represents the gold standard for diagnosing and staging Non-alcoholic fatty liver disease (NAFLD). We have previously developed automated-machine-learning based quantitation of histological features of NAFLD (fat, fibrosis, ballooning, inflammation), which provides more reproducible results compared to conventional scores. We aimed to investigate the correlation between clinical parameters and histological quantitation.

__Method:__ Liver biopsies diagnosed with NAFLD were stained with HandE and Sirius Red and were digitalized. Fat%, inflammation%, ballooning% and Collagen% were obtained using automated quantitation. They were also scored for the NASH CRN scoring system by two histopathologists. Histological results were therefore compared to anthropometric, demographic and biochemical data and fibroscan at the time of liver biopsy.

__Results:__ We analysed 246 consecutive liver biopsies. Median fat% was 2.6% (IQR 1.7-3.8) for grade 1, 15.1% (IQR 10.1-20.1) for grade 2 and 28.4% (IQR 20.2-31.9) for grade 3 steatosis (as per CRN scoring). Median inflammation% was 0.9% (IQR 0.3-1.7) for score 0, 1.1% (IQR 0.7-3.3) for score 1, 3.8% (IQR 3.1-4.1) for score 2 and 4.7% (IQR 4.4-5) for score 3 of inflammation. Median ballooning% was 4.9% (IQR 4.3-8.7) for score 0, 17.8% (IQR 13.5-24) for score 1 and 23% (IQR 20.1-32.3) for score 2 of ballooning. Median collagen was 1.3% (IQR 0.6-2) in stage 1, 2.3% (IQR 1.9-4.3) in stage 2, 5.1% (IQR 2.8-8.2) in stage 3 and 13% (IQR 5.5-20) in stage 4 of fibrosis.

Median fat% correlated with AST (Rho = 0.32, p = 0.009 on Spearman) and Controlled Attenuation Parameter (CAP) score (Rho = 0.45, p = 0.002). Median ballooning% correlated with BMI (Rho = 0.31, p = 0.03) and a β = 0.27, p = 0.05) on regression analysis (Figure 1A). Median inflammation% was significantly higher (p = 0.009, ANOVA) among Arabs (3.3%) and South-Asians (Indian, Pakistani and Bangladeshi: 3.2%) compared to other ethnic groups (Caucasians: 1.2%; Hispanics and Latinos: 1.4%, East Asians 1.4%; Blacks 0.5%) (Figure 1B). Finally, CPA correlated with age (Rho = 0.28, p = 0.001) and BMI (Rho = 0.21, p = 0.001). Among non-invasive markers, CPA correlated with NAFLD fibrosis score (Rho = 0.41, p = 0.001), FIB-4 (Rho = 0.34. p = 0.002) and liver stiffness (Rho = 0.52, p = 0.001).

__Conclusion:__ We have identified correlations between histological features and clinical characteristics that have not, to our knowledge been previously-described. Quantitation may give us novel insights into the aetiopathogenesis and disease phenotype.

__Figure:__
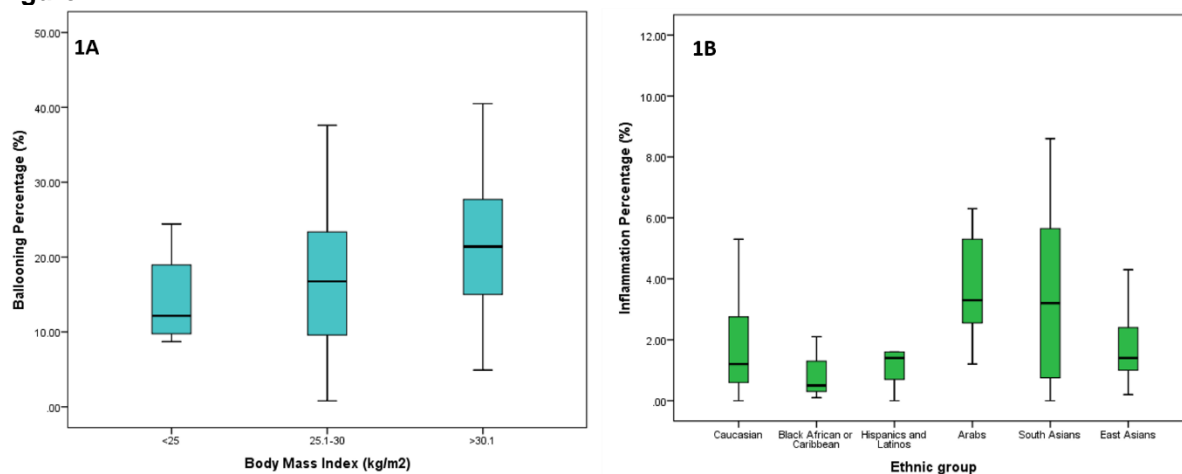
P02-14 A translational mouse model for NASH and advanced fibrosis in association with atherosclerosis

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Background and aims: Non-alcoholic steatohepatitis (NASH) is a fast-growing liver disorder in the Western world and is associated with an increased incidence of cardiovascular disease and type 2 diabetes. Animal models adequately mimicking this condition and that display both the metabolic and histological features of human NASH are scarce. We herein investigate whether Ldlr-/-.Leiden mice on a high fat diet represent a suitable NASH model.

Method: Ldlr-/-.Leiden mice were fed high-fat diets (no added cholesterol) containing lard or milk fat for 28 weeks. Effects on body weight, plasma and liver biochemical variables, liver histology, adipose tissue (inflammation) and atherosclerosis (aortic root) were assessed. Additionally, disease induction at earlier timepoints in the milk-fat group were investigated by taking a liver biopsy at t = 12 weeks and sacrifice at t = 22 weeks. The response to treatment (week 18-28) with 10 mg/kg/d FXR agonist obeticholic acid (OCA) on NASH and fibrosis was also evaluated.

Results: Both high-fat diets induced obesity, hyperlipidemia, hyperinsulinemia, and increased ALT and AST levels. Mice on both diets developed progressive macro- and micro-vesicular steatosis, hepatic inflammation and fibrosis. OCA treatment significantly reduced hepatic inflammation and fibrosis in both models. Lard-fat diet group had more severe hyperinsulinemia and adipose tissue inflammation, while milk-fat diet group had more severe hepatic inflammation with advanced bridging fibrosis (F3) in all mice after 28 weeks. Another longitudinal study with the milk fat diet revealed that after 22 weeks on the diet fibrosis was significantly induced, but primarily in F1-F2 stage with occasionally bridging fibrosis. In addition, milk-fat diet induced severe atherosclerotic lesions (primarily type IV and V based on AHA classification) in the aortic root area after 22 weeks.

Conclusion: Ldlr-/-.Leiden mice fed high-fat diets recapitulate features of the metabolic syndrome and NASH with progressive liver fibrosis and simultaneous atherosclerosis development. By adaptation of the fat content of the diet, either insulin resistance and adipose tissue inflammation (lard-based diet) or hepatic inflammation and fibrosis (milk-fat diet) can be emphasized. This represents a novel translational animal model of fibrosing NASH in association with atherosclerosis that can be used to investigate the effects of new drugs, alone (or drugs in combination).

P02-15YI Gut microbiota composition in patients with non-alcoholic fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver disease worldwide. In addition to the well-established "two-hit" theory, the alteration of gut microbiota also promotes the development of NAFLD by mediating processes of inflammation. NAFLD patients are typically characterized with small intestine bacterial overgrowth (SIBO). Hence, there is an interest in exploring the fatty infiltration as the result of the syndrome, that includes bacterial composition disturbance, with profound analysis of preventive and aggressive factors that impact on liver disease occurrence and progress. The aim of this study was to analyze the gut microbiota composition in patients with NAFLD with possible examination of aggressive and protective factors, including SIBO existence and biochemical markers.

Method: 43 patients with hyperlipidemia with average age 46.97 ± 2.53. and BMI 27.43 ± 0.74 were examined in "Medicover Ukraine" (Lviv, Ukraine). Biochemical evaluation included lipid profile, CRP, ALT, AST, GGTP, CRP, bilirubin, apolipoprotein B and A1. DNA of Bacteroidetes, Firmicutes and Actinobacteria was performed with quantitative real-time PCR (qRT-PCR), using gene-targeted primers. Ultrasound examination was proved to all patients. The criteria for fatty infiltration existence was a diffuse increase in the echogenicity of the liver parenchyma, decreased attenuation on the liver and ratio between the brightness level of the liver and the right kidney that was calculated for the hepato-renal index (HRI) determination. All subjects were examined by a lactulose breath test (LBT).

Results: The prevalence of SIBO in patients with NAFLD was 51.2%. The percent composition of microbiota included next proportions of bacteria-Bacteroidetes-16.7 ± 2.99, Firmicutes-45.3 ± 2.99, Actinobacteria-25.9 ± 1.9, Firmicutes/Bacteroidetes ratio (F/B)-6.47 ± 1.55.). Strong negative correlation between Bacteroidetes and Firmicutes ($r = -0.93$), Bacteroidetes and Firmicutes/Bacteroidetes index ($r = -0.65$) and Bacteroidetes and Actinobacteria ($r = -0.89$) was marked. Moreover, there was strong positive correlation among F/B index and triglycerides ($r = 0.42$) and ALT ($r = 0.4$). Additionally, there was middle-strong correlation between SIBO existence and Firmicutes increasing in patients with NAFLD ($r = 0.39$).

Conclusion: The prevalence of SIBO in patients with NAFLD was 51.2%. The decreasing of Bacteroidetes leads to Firmicutes and Actinobacteria increasing, with F/B growth, that provokes triglycerides and ALT level raise in patients with NAFLD. The increasing of Firmicutes is associated with SIBO presence. F/B index could be the marker of NAFLD presence, while the Bacteroidetes are potentially preventive factors for NAFLD progression.

P02-16 Propionic acid intervention in obese Ldlr-/-.Leiden mice attenuates NASH development, but negatively affects cognition

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Background and aims: There is an increasing interest to elucidate the health effects of short-chain fatty acids (SCFAs) on metabolism, obesity and brain function. Obesity is often associated with the development of non-alcoholic steatohepatitis (NASH) and cognitive impairment. We herein investigated potential health effects of the SCFA propionic acid (PA) on NASH development and brain function including cognition and behavior readouts.

Method: During 17 weeks of run-in, LDLR-/-.Leiden mice received either high-fat diet (HFD) to establish obesity or chow as control. Obese mice were matched into groups (n = 15/group) and treated with propionic acid (PA+HFD), or a reference fatty acid (caproic acid; CA+HFD), or HFD without supplements (HFD). Cognitive and behavioral effects, as well as metabolic and inflammatory risk factors, were assessed prior to and after 12 weeks of treatment. At end point, liver and adipose tissue pathology were histologically and biochemically analyzed.

Results: PA, but not reference CA, reduced body weight and this effect was independent of food intake. PA also reduced fasting insulin levels and plasma cholesterol levels relative to the start of intervention. In addition, PA reduced total and subcutaneous fat mass, but did not affect WAT inflammation. Histopathological analysis of the liver demonstrated that PA reduced macrovesicular steatosis, hypertrophy and inflammation. Consistent herewith, PA reduced the inflammatory marker serum amyloid A and tended to increase intrahepatic ketone bodies (β-hydroxybutyrate), and lowered the hepatic collagen content. PA treatment did not affect normal behavior in the open field test but mice showed impaired spatial memory, i.e. the latency to find the platform in the Morris water maze was increased. In line with these findings, synaptophysin expression in the hippocampus and vasoactivity in both the cortex and hippocampus and mitochondrial activity in the cerebellum was decreased by PA. The reference fatty acid CA exerted no effects on the above readouts.

Conclusion: Overall, PA exerted pronounced positive effects on metabolic risk factors (insulin, cholesterol, adipose mass) and attenuated the development of NASH and liver fibrosis. The observed higher levels of intrahepatic ketone bodies suggest that lipid β-oxidation is increased which may contribute to the health effects in the liver and reduced fat mass. Contrarily, PA negatively affected spatial memory, which could be a result of reduced synaptophysin expression and decreased mitochondrial function in the brain leading to impaired neurotransmitter signaling and cognitive performance.

P02-17 Comparing computerized tomography indices and liver biopsy in liver transplantation donors for hepatosteatosis

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Background and aim: Computerized tomography (CT) and liver biopsy (LB) are frequently applied in the diagnosis of steatosis in donors. In this study, we sought to investigate the efficacy of CT in the evaluation of hepatosteatosis in living donor liver transplantation (LDLT) donors in our transplantation center and the correlation of certain CT indices with LB findings.

Materials and Methods: The LDLT cases between January 2015 and December 2017 were screened retrospectively. The study participants were divided into three groups according to the degree of steatosis determined during LB as follows: grade 0: less than 5%, grade 1: 6% to 20%, and grade 2: greater than 20%. Using CT scans, hepatic attenuation value (CT^L), hepatic attenuation value and spleen attenuation value ratio ($CT^{L/S}$), and the difference between hepatic attenuation value and splenic attenuation values (CT^{L-S}) were determined and the correlations of these indices and the findings of LB were compared.

Results: Of the 60 patients (42 males, mean age: 32.4 ± 7.7 years), 43 had grade 0, 15 had grade 1, and two had grade 2 hepatosteatosis, respectively. The CT^L, $CT^{L/S}$, and CT^{L-S} cutoff values were 48.3, 1.06, and 3.2, respectively, while the sensitivity and specificity results of these cutoff values were 64.7% and 88.3%, 64.7% and 86%, and 64.7% and 86% and the area under the curve values were determined to be 0.81, 0.79, and 0.80. ($p < 0.001$).

Conclusion: CT indices are strongly correlated with LB findings. The use of these non-invasive indices can reduce the need for LB, which is an invasive procedure, as well as lessen the associated complication and cost rates. Future prospective studies are needed on this subject.

Table 1: Table 1 Demographics and CT indexes of donors according to hepatosteatosis

	Group A (n:43)	Group B (n:17)	P value	
Age	32 ± 7.9	33.4 ± 7.5	p > 0.05	
Male	25 (58.1%)	17 (100%)	**p < 0.05**	
AST	20.5 ± 6	25.5 ± 6.9	**P < 0.05**	
ALT	22.7 ± 16.8	32.9 ± 17.6	**P < 0.05**	
ALP	72.3 ± 20.7	68.4 ± 14.6	p > 0.05	
GGT	21.2 ± 13.5	44.5 ± 39.1	**P < 0.05**	
Total Cholesterol	179.6 ± 30.5	187.3 ± 23	P > 0.05	
Triglycerides	121.7 ± 64.7	126.7 ± 60	p > 0.05	
BMI	26 ± 3.8	26.8 ± 3	p > 0.05	
HOMA- IR	3 ± 2.3	4.1 ± 4	P > 0.05	
CT^L	55.5 ± 7.8	47.6 ± 6.1	**P < 0.05**	
CT^S	45.8 ± 3.4	45.4 ± 2.7	P > 0.05	
CT^{L-S}	9.7 ± 8.6	2.2 ± 7	**p < 0.05**	
$CT^{L/S}$	1.22 ± 0.2	1 ± 0.15	**P < 0.05**	

P02-18YI Transcriptomic and epigenetic characterization of a NAFLD murine model

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Background and aims: Epigenetics play an important role in the progression of non-alcoholic fatty liver disease (NAFLD). The aim of our study was to unravel how the interaction between genes and miRNAs expression could affect the development of non-alcoholic steatohepatitis (NASH) in a murine model.

Method: Fifty-two male six-weeks mice C57BL/6J were fed a HFHCC diet (40% Kcal fat, 1% cholesterol and 42g/L glucose/fructose in drinking water) (n = 40) or standard diet (n = 12) being sacrificed at 13, 26, 39 and 52 weeks. Anatomorphological features, histological, biochemical and metabolic parameters were measured. Liver RNA was extracted and the transcriptomic and epigenetic profile were studied by ClarionS and miRNA4.0 arrays including >22, 000 transcripts and >3, 000 miRNAs (mature and pre-miRNA) (ThermoFisher, CA, USA). The interaction was evaluated by using TAC software (ThermoFisher, CA, USA).

Results: HFHCC diet induced NASH in mice, characterized by a gain in weight and BMI, macro-microvesicular steatosis (>90%), inflammatory foci at lobular, portal and periductal levels, followed by presence of hepatocyte degeneration (ballooning) and moderate fibrosis. The model also showed a progressive increase in the hepatic, lipidic and glucidic parameters. Besides, we also detected several foci of cellular alterations in >40% animals and nodules in >20%. The transcriptomic gene enrichment analysis allowed us to identify changes in pathways similar to human NASH. At early stages, changes in lipid metabolism, proliferation and inflammatory signaling were observed. Nevertheless, advanced stages showed an increase in oxidative stress, senescence and innate immune response pathways. Besides, we found upregulation of several pluripotency-related factors such as Sox2, Nanog and Klf4 at 52 weeks. In comparison to control, NASH animals showed up to 28 genes differentially expressed ($p < 1 \times 10^{-8}$;fold ± 10;FDR<0.05) Similarly, the epigenetic profile revealed 26 miRNAs with differences ($p < 0.001$;fold ± 2;FDR<0.05). Finally, by analyzing the target gene prediction of miRNAs we could confirm the interactions miRNA-genes (miRNAome).

Conclusion: HFHCC model comprises the main clinical and histological characteristics of human NASH, including slow progression to fibrosis and hepatocellular carcinoma, covering the needs in the field for future preclinical studies. Besides, by deciphering the miRNAome of NAFLD new biomarkers and therapeutic targets could be developed and detected. In this sense, we found that overexpression of four miRNAs (miR-200b, miR-199a, mir181b and miR34a), through regulating several genes, could play a role in NAFLD pathogenesis.
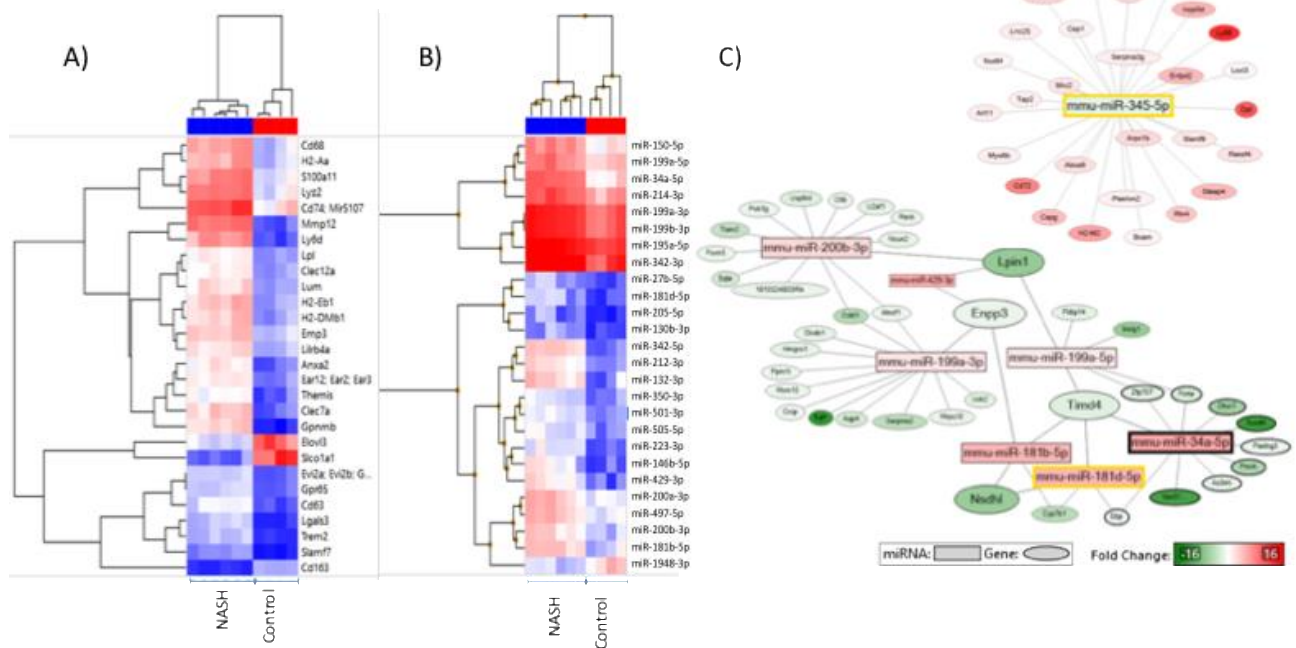
P02-19YI Osteopontin deficiency promotes liver senescence mediating the onset of non-alcoholic fatty liver disease during aging

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Background and aims: Osteopontin (OPN), a cytokine part of the senescence-associated secretory phenotype, increases in different stages of non-alcoholic fatty liver disease (NAFLD). During aging prevalence of metabolic diseases and the risk for NAFLD rises. p53 activation contributes to senescence but can also reduce lipid accumulation. Our aims were: **1)** To investigate the role of OPN in the aging-related senescence induction and its contribution to NAFLD development. **2)** To elucidate the role of p53 regulating OPN in senescence and NAFLD

Method: Serum OPN was measured in a cohort of normal liver (NL) humans. OPN-deficient mice (OPN-KO) and their controls (WT) of 3, 10 and 20 months-old (mo) were used. To identify the dysregulation in metabolic pathways, serum and liver lipid concentration, metabolic fluxes and bile acid (BA) analysis ware performed

Results: In NL individuals, serum OPN levels correlated positively with age. In WT mice, serum and liver levels of OPN increased with age, showing a sharp increase at 10 mo. Together with OPN, liver p53 levels increased along with age. Senescence markers, liver concentration of neutral lipids and their *de novo* synthesis increased in 10 mo OPN-KO mice. This phenotype was associated with decreased GRP78 chaperone levels and activation of ER stress. Moreover, in 10 mo OPN-KO mice, analysis of biliary metabolism showed increased CYP7A1 levels and 12a-hydroxy-BAs/non-12a-hydroxy-BAs ratio, a marker of metabolic dysregulation. In 20 mo, damage markers and higher fibrosis showed a worse progression of the liver disease with age when OPN is deficient. When senescence was induced in HepG2 cells, OPN levels raised. Moreover, treatment with recombinant OPN reduced the effect of the senescence inducer palbociclib, making cells more resistant to senescence. In addition, knockdown of p53 in HepG2 cells reduced OPN levels and prevented its increase when senescence was induced. The same phenotype was observed in Hep3B cells, which are p53-null. Besides, p53-KO mice were unable to reach the increase of OPN liver levels when fed a high fat diet

Conclusion: OPN is required to prevent the aging-related liver metabolic complications. Lack of OPN induces senescence, which will promote a decrease in GRP78 and activation of ER stress. Thus, inducing dysregulation of lipid metabolism and worsened progression of NAFLD during aging. The results suggest that p53-induced OPN expression mediates a link between cellular senescence and lipid metabolic dysregulation in liver during aging

P02-20 Actions of the protease fibroblast activation protein alpha (FAP) on collagens and FGF21 and roles in chronic liver injury

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Background and aims: FAP is a unique post-proline peptidase that is greatly upregulated in activated stellate cells in human liver fibrosis. Circulating FAP rises and falls with liver fibrosis severity. We aim to better understand NAFLD pathogenesis by delineating actions of FAP. The major FAP substrates are collagens, the starvation hepatokine FGF-21 and neuropeptide Y.

Method: Our FAP gene knock-in (gki) mouse, which lacks FAP enzyme activity was fed atherogenic high fat high sucrose diet (HFD) for 17 weeks, or provided thioacetamide to induce fibrosis. Embryonic fibroblasts were analysed in SILAC and TAILS proteomics methods. FAP enzyme activity was measured in sera from NAFLD patients, using an in-house assay.

Results: With HFD, compared to WT mice, FAPgki mice had less insulin resistance, pancreatic and plasma insulin, glucose intolerance, micro-vesicular steatosis, liver lipid, serum alanine transaminase, circulating LDL cholesterol and islet area. FAP deficiency greatly increased intrahepatic non-esterified free fatty acids and increased ACC, indicative of increased lipolysis and β-oxidation. CD36 immunostaining was lower in FAPgki hepatocytes. Concordantly, lipogenic genes (*Pparγ, Gck, Acc and Fasn*) and hepatic triglyceride and fatty acid uptake genes (*Cd36, Apoc3, Ldlr*) were downregulated in FAPgki livers. FAP action may involve intrahepatic FGF-21, as it was increased in FAP deficient compared to WT mice. In the fibrosis model, significantly less Sirius red staining and leukocyte clusters were evident in FAPgki livers.

Proteomics using fibroblasts showed that FAP modulates the abundance of proteins involved in ECM remodelling, including collagens 1, 3 and 5, ECM-1, lysyl oxidases and fibronectin in vitro. Substrate cleavages generally occurred after Gly-Pro.

Circulating levels of FAP were associated with fibrosis severity, insulin resistance and prothrombin time.

Conclusion: The human data associates FAP with liver disease pathogenesis. This study is the first to demonstrate that specific genetic ablation of FAP activity, which mimics a specific potent inhibitor, is protective of diet-driven metabolic defects and fibrosis in mice. Our data indicates that a suitable FAP inhibitor may have potential as a therapy for insulin resistance, steatosis and liver fibrosis.

P02-21 The Vanin 1-Cysteamine pathway regulates immune tolerance upon lipid-induced oxidative stress in non-alcoholic fatty liver disease

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Background and aims: Lipid-induced oxidative stress is considered a critical factor in the progression of non-alcoholic fatty liver disease (NAFLD). Vanin-1 (VNN1) is a pantetheinase that hydrolyses Vit B5 into cysteamine as part of the co-enzyme A pathway and has been reported to modulate hepatic triglyceride levels and to be protective against acetaminophen induced hepatoxicity. This study aims to investigate the role of Vanin-1/cysteamine in the progression of NAFLD.

Method: *VNN1* rs4897612 genotype was assessed in a cohort of 465 patients with histological proven NAFLD. Vnn1 -/- knock-out and wild type C57BL/6 mice were fed a methionine-choline deficient diet for 6 weeks. Cysteamine intervention treatment was started at week 2. The human hepatoma HepG2 and the myeloid Mono-Mac6 cell lines were challenged with oleic, linoleic and palmitic acid *in vitro* with or without cysteamine. Murine hepatoma Hepa1-6 cells were co-cultured with bone marrow-derived macrophages in the presence of lipids ± cysteamine. Read-outs were done using histochemical stainings, proliferation, ROS and Seahorse assays, ELISA and qPCR.

Results: Multivariate analysis showed that the *VNN1* variant rs4897612 was associated with an increased risk of advanced hepatic fibrosis F3-4 ($p = 4.9*10^{-3}$) independent from age, BMI, T2DM or *PNPLA3* rs738409 variant in a cohort of 465 patients. MCD-fed Vnn1 -/- mice showed significantly more hepatic lipogranuloma, sinusoidal fibrosis and progenitor cell activation, together with reduced glutathione levels. Oral submission of cysteamine started at week 2 of the MCD diet reduced the liver weight and the hepatic oxygen consumption rate. *In vitro* cysteamine significantly reduced lipid-induced oxidative stress by increasing glutathione levels and reducing ROS. Moreover, cysteamine induced the translocation of NRF2 from the cytoplasm to the nucleus, resulting in increased levels of AKR1B10 and p62-postive phagosomes. In addition, in murine co-culture systems cysteamine reduced *Tnfa* and *Il6* expression.

Conclusion: Our results suggest that, under the influence of modifiers including Vanin-1, cysteamine levels contribute to immunetolerance and reduce mitochondrial stress and the inflammatory potential in metabolic diseases like NAFLD.

P02-22 Risk stratification of patients with non-alcoholic fatty liver disease in primary care using a reflex testing algorithm of FIB-4 and enhanced liver fibrosis score

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Background and aims: Patients with non-alcoholic fatty liver disease (NAFLD) and advanced fibrosis or cirrhosis have elevated liver-related and cardiovascular mortality and may benefit from management in a secondary care setting. As NAFLD is asymptomatic, and many have normal liver enzymes, the majority of these patients are missed in routine practice. We report the first year of data from our primary care NAFLD algorithm.

Method: Oxford University Hospitals NHS Foundation Trust and Oxfordshire Clinical Commissioning Group introduced this primary care NAFLD stratification pathway in November 2017. All adult patients with suspected NAFLD, regardless of liver enzyme results, are eligible for inclusion. Investigations are requested electronically by primary care. The algorithm utilises FIB-4 measurement, with subsequent reflex testing of ELF in patients with intermediate FIB-4 results. Patients with high risk FIB-4 (>2.67), or intermediate FIB-4 (1.30-2.67) with elevated ELF score (>9.5) are advised to be referred to a specialist Metabolic liver clinic. Patients aged <35 years, in whom FIB-4 may underestimate fibrosis risk, directly undergo ELF testing with a score >9.0 indicating elevated fibrosis risk. Advanced fibrosis and cirrhosis were diagnosed based upon histology, radiology or supportive Transient Elastography (≥8 kilopascals for advanced fibrosis or ≥11.5 kilopascals for cirrhosis) as part of a global assessment.

Results: Between November 2017 and November 2018, 457 patients were investigated. Of these, 338 (74%) of patients had low risk biomarker results and could avoid hospital referral. This includes 294 patients (64.3%) with low risk FIB-4 scores, and 44 patients (9.6%) with intermediate FIB-4 but low risk ELF score. Overall, 80% of patients with elevated risk scores were seen in the secondary care clinic. 35.1% of clinic referrals had advanced fibrosis on global assessment, including 14.1% diagnosed with cirrhosis (of whom 3 patients had oesophageal varices). Liver biopsy was undertaken in 31 patients. Fibrosis stages (Brunt classification) were F0 fibrosis in 2 patients, F1 in 6, F2 in 9, F3 in 8 and F4 in 6.

Conclusion: We have demonstrated that a NAFLD primary care risk-stratification algorithm using reflex biomarker testing with FIB-4 and ELF score is feasible to implement and results in the diagnosis of a significant number of patients with advanced fibrosis and cirrhosis, including those with early portal hypertension.

Figure:

Figure 1
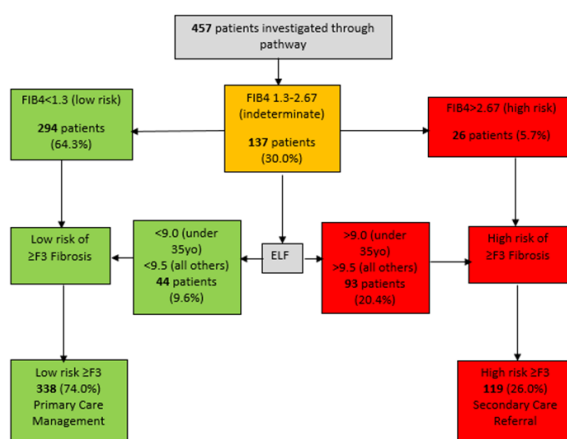
P03-03 Estimated GFR is associated with PNPLA3 risk variant p.I148M in patients with non-alcoholic fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) belongs to the most common liver diseases, and the *PNPLA3* p.I148M variant is known for its steatogenic and fibrogenic effects. Few longitudinal studies for *PNPLA3* and impaired kidney function exist. Here we investigate whether the *PNPLA3* p.I148M has an impact on kidney function in the context of NAFLD.

Method: Prospectively we recruited patients in our liver and diabetes outpatient clinics. NAFLD was quantified non-invasively by controlled attenuated parameter (CAP) and liver stiffness measurement (LSM). Patients were stratified using CAP ≥248 dB/m and LSM ≥9.2 kPa as cut-offs for significant steatosis and fibrosis, respectively. Liver diseases other than NAFLD were excluded. All patients filled in a battery of Health related Quality of Life (HrQoL) questionnaires. Laboratory analyses including estimated glomerular filtration rate (eGFR) were performed with standard clinical-chemical methods. *PNPLA3* p.I148M was genotyped using TaqMan assays.

Results: Among 107 patients (57 women, age 60 ± 13 years) 84% presented with type 2 diabetes (DM2) and 76% with significant steatosis. eGFR was neither associated with the presence of fatty liver nor fibrosis, but both self-reported DM2 and cardiovascular diseases affected renal function in the whole cohort (all $p < 0.05$). Of note, patients with NAFLD carrying at least one *PNPLA3* risk allele showed significantly ($p = 0.023$) lower eGFR than those with the wild-type genotype. The association between *PNPLA3* p.I148M and eGFR was apparent already in heterozygotes ($p = 0.047$) but was absent in patients with DM2 without fatty liver ($p > 0.05$). Lower eGFR was also associated with impaired HRQoL according to Short Form Health Survey and Modified Fatigue Impact Scale in the NAFLD group (all $p < 0.05$), but this effect did not appear in the control group ($p > 0.05$).

Conclusion: eGFR seems to be associated with the *PNPLA3* risk variant in elderly patients with NAFLD. Lower eGFR is also associated with impaired HrQoL in this group. Our results emphasize that NAFLD is a multisystemic disease and support a specific role of genetic risk factors modulating the liver-kidney-axis in the context of NAFLD.

P03-04YI Cannabis consumption prevents hepatic steatosis in psychosis patients

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Background and aims: The endocannabinoid system regulates feeding behaviors and energy and metabolic homeostasis. Recent evidence indicates a protective effect of cannabis consumption in psychosis patients on weight gain and related metabolic alterations, which are frequently present in these patients as a result of antipsychotic treatment. However, there are no previous studies on the likely effect of cannabis on liver steatosis.
We aimed to explore if cannabis consumption had an effect on hepatic steatosis in psychosis patients.
Method: A total of 390 patients were evaluated at baseline and after 3 years of initiating the antipsychotic treatment. Anthropometric measurements, blood test and cannabis use were collected at both time points. Liver steatosis and fibrosis were evaluated through validated clinical scores (Fatty Liver Index [FLI], Fibrosis-4 [FIB-4], and NAFLD Fibrosis Score [NFS]).
Results: At baseline, 150 patients (38.5%) were cannabis users. At 3-year follow-up, cannabis users presented significantly lower body mass index, abdominal perimeter and FLI score than nonusers (11.8 vs 40.3; $p < 0.001$). After excluding patients with high alcohol consumption (n = 40), there was a lower percentage of cannabis users with FLI >60 than non-users (6.7% vs 26.1%; $p = 0.007$). Moreover, patients maintaining cannabis consumption after 3 years presented the smallest increment in FLI overtime, which was significantly smaller than the increment in FLI presented by discontinuers ($p = 0.022$) and never-users ($p = 0.016$). None of the patients presented a FIB-4 and NFS suggestive of advanced fibrosis, so we were unable to analyze the association between fibrosis and cannabis consumption.
Conclusion: Cannabis consumption may produce a protective effect against liver steatosis in psychosis, probably through the modulation of antipsychotic-induced weigh gain, although a direct effect of cannabis on the liver tissue has not been ruled out.

P03-05YI Triple targeting of nuclear receptors protects against diet-induced NAFLD in mice

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are driven by deregulated lipid metabolism and inflammation. As key regulators of hepatic metabolism, bile acid-activated receptors impact on NAFLD, supporting the use of agonists to treat disease. To this end, ligands of the farnesoid X receptor (FXR) and G protein-coupled receptor 5 (TGR5) are promising, although efficacy is still limited and accompanied by side effects, which may be offset through complementary approaches. Because we have shown that miR-21 ablation protects mice from NAFLD, mainly through peroxisome proliferator-activated receptor α (PPAR- α) de-repression, here we aimed to elucidate whether INT-767, a dual FXR and TGR5 agonist, alone or in combination with miR-21 silencing, could synergize in ameliorating NAFLD and its progression to hepatocellular carcinoma.

Method: C57BL/6N mice were fed a high fat choline deficient (HFCD) diet for 14 or 24 weeks, supplemented with or without INT-767, and/or injected with antagomiR-21 or a scrambled antagomir (control). Liver samples were collected and processed for histology. Expression of pro-inflammatory, pro-fibrogenic, lipid and cholesterol metabolism genes were investigated through qRT-PCR.

Results: 14-week HFCD-fed mice developed NASH with minimal fibrosis, whereas 24-weeks-fed animals already displayed significant fibrosis and, notably, pre-neoplasic nodules. Animals in both time points further exhibited evidence of increased insulin sensitivity and liver damage (AST and ALT). Noteworthy, INT-767 and antagomiR-21, alone or in combination, prevented disease progression at both time points. Particularly, upregulation of pro-inflammatory markers IL-1β, TNF-α, IL8, NLRP3, TL4 were significantly reverted by INT-767 and antagomiR-21 alone and, more strikingly, by both in combination. In agreement with histological analysis, pro-fibrogenic markers were slightly increased in HFCD-diet fed mice for 14-weeks. At 24-weeks, upregulation of liver collagen1α1, α-SMA, TGF-β was prevented in INT-767 and/or antagomiR-21 treated animals. Finally, HFCD diet-induced downregulation of SHP, PPAR-α and MCAD was also inhibited by both INT-767 and antagomiR-21 alone, and further by both in combination.

Conclusion: In conclusion, while either INT-767 or antagomiR-21 alone ameliorate the NAFLD phenotype at distinct disease stages, combination of both treatments appears to afford higher therapeutic effects. A better elucidation of the complementary signalling pathways targeted by INT-767 and antagomiR-21 will help to establish the validity and significance of this putative therapeutic approach in preventing NAFLD development and its progression towards more severe stages.

P03-06 Mitochondrial DNA analysis in NASH patients

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Background and aims: Mitochondrial dysfunction (i.e., respiratory chain deficiency in a more particular sense) plays a key role in the physiopathology of non-alcoholic steatohepatitis (NASH), regardless of the initial cause of the condition. Mitochondrial dysfunction can also lead to apoptosis or necrosis, depending on the cell energy status. while reactive oxygen species (ROS) and lipid peroxidation products also enhance the generation of several cytokines (TNF-alpha, TGF-beta, Fas ligand), which play a key role in cell death, inflammation and fibrosis. In our study, we investigated the mitochondrial DNA (mtDNA) copy number, damage, repair and degradation in peripheral blood mononuclear cells (PBMCs) of patients with NASH and compared the obtained results with those in healthy subjects.

Method: Total genomic DNA was isolated from PBMCs of 35 biopsy proven NASH patients and 40 healthy subjects before, immediately after, and 3h after an exposure to H2O2. An evaluation of mtDNA copy number was performed by the real-time PCR and $2^{-\Delta ct}$ methods. The semi-long run real-time PCR technique was used to estimate the number of mtDNA lesions.

Results: The baseline mtDNA copy number did not differ between the cells from healthy subjects and the cells from the NASH patients. After a 10-min challenge with hydrogen peroxide (H2O2), the PBMCs from the NASH patients exhibited slower changes in the copy number, indicating a lower efficiency of mtDNA degradation, when compared to the controls. Moreover, a significantly higher number of mtDNA lesions was found in the NASH patients at baseline, as well as at the other experimental time points. mtDNA lesions were also elevated in the cells from the NASH patient immediately after the exposure to H2O2. An Induction of oxidative stress had no significant influence on the cells of the controls.

Conclusion: Our preliminary results showed that the impaired repair and degradation of mtDNA could be involved in the pathophysiology of NASH.

Our preliminary results provided a fairly reliable evidence for the role which both, the impaired repair and degradation of mtDNA could play in the pathogenesis of NASH.

P03-07 High-throughput sequencing identified miR-193a as a potential biomarker of non-alcoholic fatty liver disease activity

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Background and aims: The current gold standard of non-alcoholic fatty liver disease (NAFLD) diagnosis is based on histological scoring of liver biopsies. Disease activity can be graded using the NAFLD activity score (NAS), calculated as the sum of Kleiner inflammation, ballooning and steatosis scores. Liver biopsies carry inherent risks and so circulating biomarkers are needed to circumvent the requirement for such invasive procedures. MicroRNAs (miRNAs) are small (~22 nt) non-coding RNA molecules that post-transcriptionally regulate gene expression and are known to be expressed in serum. Circulating miRNAs have been characterised as diagnostic biomarkers for a range of diseases. Accordingly, we sequenced over 2, 000 serum miRNAs in a discovery cohort of patients across the NAFLD spectrum to establish a profile of circulating miRNAs from which novel disease biomarkers could be identified.

Method: miRNA libraries, using 15 µl serum for each of 183 NAFLD patients and ten healthy controls, were generated by HTG EdgeSeq and sequenced by Illumina NextSeq. Limma in the R software environment was used to perform analyses on the data. Data were normalised and corrected for batch effects. MiRNAs with a mean counts per million of ≥100, a log2 fold-change (logFC) of ≥0.3 and an adjusted p value of ≤0.05 were classified as differentially expressed.

Results: Seven miRNAs were differentially expressed in severe disease activity (NAS5-8) relative to mild (NAS1-4), with miR-193a being the most significant (logFC = 0.68, $p = 3.0 \times 10^{-07}$, AUROC = 0.71). Additionally, miR-193a was the most significant (logFC = 1.5, $p = 3.5 \times 10^{-10}$, AUROC = 0.94) of 121 differentially expressed miRNAs above a more stringent logFC threshold of ≥1 in NAFLD patients relative to controls. Distilling NAFLD into steatosis and non-alcoholic steatohepatitis (NASH) with fibrosis stage (F0-F4) confirmed a consistent and significant upregulation of miR-193a in each of the classifications relative to the controls.

Conclusion: We have identified a potentially clinically significant differentially expressed circulating miRNA, which appears to primarily reflect NAFLD grade and activity. Quantification of miR-193a may facilitate non-invasive NAFLD diagnoses. This, along with other significantly differentially expressed miRNAs, is currently being validated in a subset of samples using qPCR.

P03-08 BTT-105 ameliorates non-alcoholic steatohepatitis on diet induced animal models and attenuated lysophosphoatidic acid induced hepatic stellate cell activation

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Background and aims: BTT-105 is a novel synthesized vitamin E derivative, which reportedly is potent Nrf2 activator. BTT-105 was well tolerated and rapidly absorbed in previous phase I study. We investigated the effects of BTT-105 on non-alcoholic liver disease (NAFLD) and mode of action of BTT-105 using different types of diet induced NAFLD models

Method: *In vivo*: Seven months high fat diet and four months methionine choline deficiency diet induced animal NAFLD models were used. Pre-study liver biopsy performed in high fat diet model at 4 months. Animals ≥3 NAS (NAFLD activity score) included using pre-study liver biopsy at 4 months and randomized into three groups (control, low- and high dose). At the end of the experiment (seven months in high fat, and three months in MCD model), histological assessment, metabolomics analysis and RNA sequencing performed. *In vitro*: GPCR functional assay performed. TGF-β, TIMP-1, collagen, and αSMA level were analyzed in activated stellate cell using TGF-β, and lysophosphatidate (LPA). Changes of retinoic acid receptor-α expression evaluated after BTT-105 treatment in activated stellate cell.

Results: BTT-105 attenuated lobular inflammation and hepatic fibrosis in seven months high fat model. ColA1, and TIMP-1 expression decreased, and MMP-9 expression increased in BTT-105 treatment group compared to control group. NAS as well as lobular inflammation decreased in BTT-105 group compared to control in four months MCD diet animal model. Glutathione, retinol, and PPAR signaling pathway activated after BTT-105 treatment in RNA sequencing analysis. qRT-PCR analysis reconfirmed the increasing GSTA1, Gstm1, and Gstm3 gene expression in BTT-105 treatment group. Retinoic acid receptor expression increased in BTT-105 group. GPCR functional assay showed BTT-105 potentially acts as EDG7 (LPA) antagonist. LPA activated stellate cell, and increased αSMA and in LX-2 cell (hepatic stellate cell line). BTT-105 inhibited stellate cell activation by TGF-β and LPA treatment. BTT-105 increased retinoic acid receptor-α, and decreased αSMA expression in LX-2 cell.

Conclusion: BTT-105 attenuated hepatic inflammation and fibrosis in different two NAFLD model. BTT-105 is waiting for Phase IIa clinical trial

P03-09 Saroglitazar, a potential treatment for Non-Alcoholic Fatty Liver Disease/Non-Alcoholic Steatohepatitis: Preliminary evidence from pre-clinical, clinical and real world studies

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Background and aims: Saroglitazar, a novel dual peroxisome proliferator activated receptor alpha/gamma agonist, is approved for diabetic dyslipidaemia in India since 2013. Currently, it is in clinical trials for non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH) indications in the USA, Mexico, and India. While the data from these trials is awaited for a clinical confirmation of its efficacy in NAFLD/NASH, preliminary evidence from pre-clinical, clinical and real world studies indicate that it can be a potential treatment based upon reduction in serum alanine aminotransferase (ALT) levels. We reviewed two preclinical studies, a phase-2 clinical trial and three real world clinical studies that were investigator initiated observational studies conducted by clinicians across India.

Method: In the preclinical DIAMOND™ NASH mice model, histology, laboratory indices, molecular and metabolomics markers were studied. Quantitative whole body autoradiography (QWBA) was evaluated in male Wistar Hanover (WH) and male Long Evans (LE) rats in the other animal model. The phase-2 clinical trial of 12 weeks duration for Saroglitazar 4mg was conducted in 32 patients with biopsy proven NASH along with ALT >1.5 times the upper limit of normal. The real world clinical studies Joshi et al (2016), Saboo et al (2015), and Goyal et al (2019) evaluated the effects of Saroglitazar 4 mg on ALT levels in patients with diabetic dyslipidemia also diagnosed with NAFLD.

Results: In the DIAMOND™ NASH mice model Saroglitazar increased insulin sensitivity, anti-oxidant signalling, gluconeogenic drive and improved NASH histology. Whereas, in the other model following oral administration of ^{14}C Saroglitazar at nominal dose of 4 mg/kg (~200μCi/kg radioactive dose) in the QWBA, the radioactivity persisted in the liver through the last sampling time of 72 h post-dose in WH rats and through 168 h post-dose in LE rats indicating Saroglitazar is hepatotropic. In the phase-2 clinical trial of 12 weeks duration, Saroglitazar 4 mg significantly reduced ALT levels (baseline to week-12: -53.70%) in 32 patients with NASH [Fig1 (A)]. And also, in the three real world clinical studies, Saroglitazar 4 mg significantly reduced ALT levels from baseline to week-24 (Fig1 (B) and Table 1).

Conclusion: The preliminary evidence from the preclinical, clinical and the real world studies indicates that Saroglitazar can be a potential treatment for NAFLD/NASH.

Fig 1(A): Phase-2 Clinical Trial of NASH: Change in ALT level (U/L) from baseline to week-12 with Saroglitazar 4 mg use

	Baseline	Week 12
ALT	95.9	44.4

(-53.70 %)
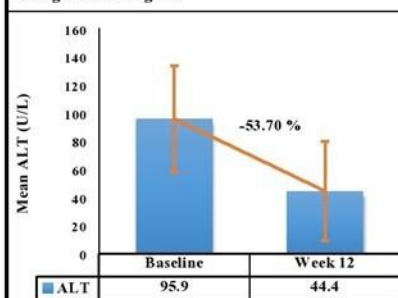
(B): Real World Clinical Studies: Change in ALT level (U/L) from baseline to week-24 with Saroglitazar 4 mg use

(b1) Study 1: Joshi et al. (2016)

	Baseline	Week 24
ALT	89	21

(-76.40 %)
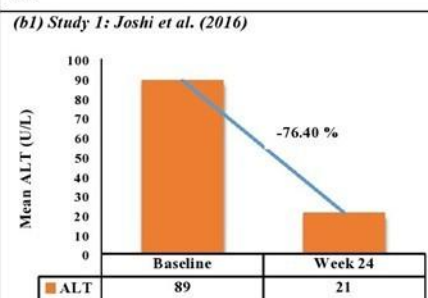
Table 1: Change in ALT level (U/L) from baseline to week-12 and week-24 with Saroglitazar 4 mg use

Study	N	Baseline (M±SD)	Week 12 (M±SD)	Week 24 (M±SD)
Clinical Trial of NASH				
Phase 2	32	95.9 ± 37.7	44.4 ± 35.4	-
Real World Clinical Studies				
Joshi et al. (2016)*	221	89.0	-	21.0
Saboo et al. (2015)	31	64.1 ± 6.2	-	28.7 ± 3.2
Goyal et al. (2019)	84	98.0 ± 32.0	-	34.0 ± 14.0

Abbreviation: M: Mean, SD: standard deviation
Note: For * study, data for standard deviation was not available.

References:
(1) Phase 2 Study (data on file)
(2) DIAMOND™ mice model (data on file; presented at AASLD 2018)
(3) Joshi et al. https://www.aace.com/files/late-breaking-abstracts-2016.pdf. P 331. Accessed 10 May 2019.
(4) Saboo et al. https://www.abstractsonline.com/pp8/#!/3699/presentation/9937. Accessed 10 May 2019.
(5) Goyal et al. Hepatol Int (2019) 13 (Suppl 1):S1–S266. Abstract #373 – S204. https://doi.org/10.1007/s12072-019-09936-5.

(b2) Study 2: Saboo et al. (2015)

	Baseline	Week 24
ALT	64.1	28.7

(-55.23 %)
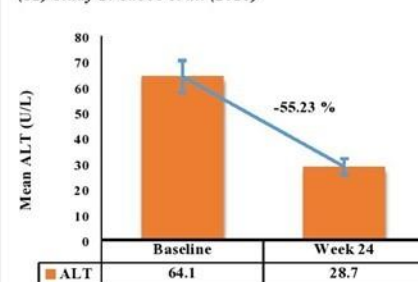
(b3) Study 3: Goyal et al. (2019)

	Baseline	Week 24
ALT	98	34

(-65.31 %)
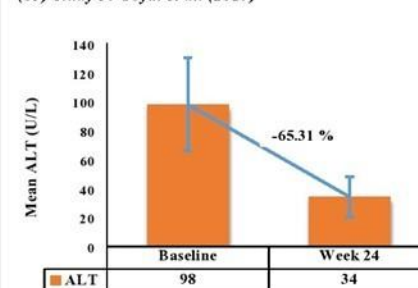
P03-10YI Validation of a simple 2 step strategy involving FIB4 and mre in evaluation of non-alcoholic fatty liver disease

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Background: Non-alcoholic fatty liver disease (NAFLD) is estimated to affect 1/3rd of the world's population. Screening strategies for identification of NAFLD in high risk population need to be simple and effective. Liver fibrosis is the single most important factor identified to impact the adverse outcome in NAFLD but it is unrealistic doing liver biopsy for assessing patients with NAFLD and those at risk for progression. Magnetic Resonance Elastography (MRE) has emerged as the best non-invasive alternative modality to liver biopsy in assessment of NAFLD. Amongst the various bio-markers FIB4 has been validated against liver biopsy as a simple easy to use marker in assessment of fibrosis. The current study hereby aimed at validating a simple 2 step strategy involving MRE and FIB4 for assessment of NAFLD.

Methods: The study samples were divided into a derivation cohort and validation cohort. The derivation cohort included two groups; first group was (N = 23) patients with NAFLD and liver fibrosis diagnosed on liver biopsy and second group was healthy liver donors for liver transplants who underwent liver biopsy (N = 50). All underwent MRE (GE Healthcare, USA). The liver stiffness on MRE and FIB4 was assessed and compared between the two groups and AUROCs were calculated. The cut offs values ofFIB4 and MRE were than prospectively evaluated in the validation cohort of 195 patients with NAFLD.

Results: The results of the comparison in derivation cohort are mentioned in the table

Derivation cohort	Fibrosis score 0 Mean ± SD	Fibrosis score 1-4 Mean ± SD	p value
Liver stiffness (Kpa)	2.15 ± 0.32	7.68 ± 3.51	<0.0001
FIB-4	0.54 ± 0.23	6.502 ± 11.373	0.0004

In derivation cohort, a FIB4 ≥1.0 showed 100% sensitivity and95% specificity for presence of fibrosis. MRE liver stiffness of ≥2.85 Kpa had 91.3% sensitivity and 80% specificity for Fibrosis score 1-4. In the validation cohort the negative predictive value of FIB4 for liver fibrosis was 85% while the positive predictive value was 23%. Using sequential FIB4 and MRE correct diagnosis was made in 184 out of 195 (94.4%).

Conclusion: FIB4 is accurate for ruling out fibrosis in patients with NAFLD. MRE reliably identifies patients with fibrosis. A 2 step strategy of FIB4 and MRE may be used to identify fibrosis in NAFLD at the community level.

P03-11YI fasting refeeding HFD mice accumulate hepatic lipid and develop metabolic dysfunction which control by NQO1 enzymatic action

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Background and aims: Because of unhealthy lifestyles, a large number of people are suffering from hepatic lipid accumulation and non-alcoholic steatohepatitis. Fasting-Refeeding with high fat diet (F/R HFD) promotes the development of hepatic steatosis and dysfunction in mice, but the effect in human is still unknown. NADH-quinone oxidoreductase 1 (NQO1) modulates intracellular NAD^+ levels which plays a fundamental role in hepatic lipid or energy metabolism, and a decontrolled NAD^+ levels is implicated in metabolic syndrome. We hypothesized that the effect of β-lapachone (β-Lap), a known cellular NAD^+ modulator that provides therapeutic effects on F/R HFD mouse model of hepatic metabolic dysfunction by the pharmacological enzymatic action of NQO1.

Method: In this study, we designed to understand F/R processing to a meal, 24 h refeeding with HFD (60% of total calories from fat) after 24 h fasting in healthy mice for 12 weeks results in hepatic damage assessed by hepatic morphology, biomarkers, ROS levels, LKB1/AMPK/ACC and p53/SIRT1 signalling.

Results: Our results show that lipid synthesis is more enhanced in liver and free fatty acid, triglyceride, FAS and SREBP-1 are notably increased by F/R HFD than regular HFD-fed mice. Additionally, the acetylated NF-κB is more triggered as a result induces the proinflammatory mediators and hepatic ROS along with fibrotic markers in liver tissue of F/R HFD mice. However, β-Lap attenuates hepatic steatosis, oxidative stress, moderate fibrosis and systemic inflammation in F/R HFD mice by reduction of the acetylating of NF-κB-p65 and p53. Furthermore, we confirmed that β-Lap inhibited PARP hyperactivation thus restored the cellular NAD^+ levels. Consistent with restored NAD^+ levels, SIRT1 activity and its expression and LKB1/AMPK/ACC phosphorylation returned to control levels in mice.

Conclusion: This study is the first to demonstrate that enzymatic action of NQO1 has a hepatoprotective effect that is mediated by F/R HFD via modulation of cellular NAD^+. Herein, our data give strong evidence that β-Lap could be a novel therapeutic approach for the prevention of hepatic metabolic damage from F/R HFD through modulation of intracellular NAD^+ levels via NQO1 enzymatic action.

Figure:
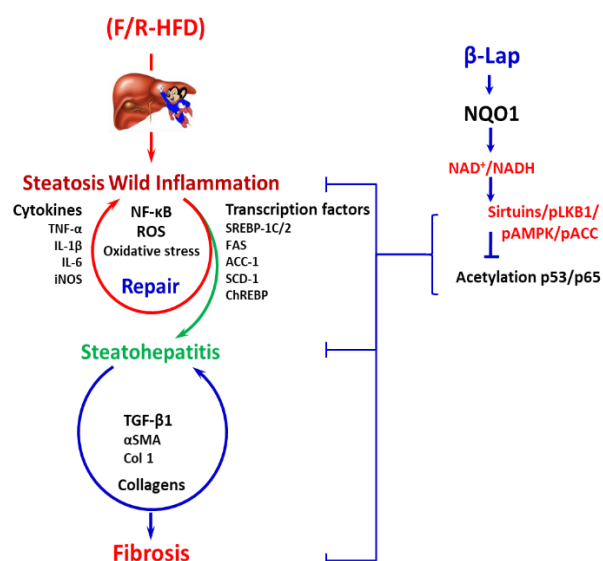
P03-12YI HSD17B13 and PNPLA3 gene variants exert opposite effects on non-alcoholic fatty liver phenotypes: results from the "real life" FLAG cohort

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a prevalent condition commonly encountered among overweight and obese individuals. The *PNPLA3* p.I148M variant represents a major genetic determinant of fatty liver disease progression. Recently the loss-of-function *HSD17B13* polymorphism rs72613567 was reported to be hepatoprotective in patients with chronic liver diseases (Abdul-Husn et al. *NEJM* 2018). Here we investigate the effects of these two gene variants on fatty liver phenotypes in a "real life" cohort of German NAFLD patients.

Method: All patients included in the analysis were recruited within the Fatty Liver Assessment in Germany (FLAG) program, a multicenter cohort study covering private and public outpatient clinics. The *PNPLA3* p.I148M and the *HSD17B13* rs72613567 polymorphisms were genotyped using allelic discrimination assays. The control cohort comprises 174 healthy individuals. The effects of both variants on patients' phenotypes were analysed in contingency tables and regression analyses.

Results: Overall, the study cohort comprised 475 individuals (255 men) with NAFLD. The *PNPLA3,* but not the *HSD17B13*, polymorphism deviated significantly ($p < 0.001$) from Hardy-Weinberg equilibrium (HWE) in the entire FLAG cohort due to overrepresentation of the prosteatotic risk allele. The *PNPLA3* p.I148M variant was more prevalent among FLAG patients as compared to healthy controls and increased the risk of developing NAFLD (common OR = 2.47, $P = 5 \times 10^{-09}$). It also correlated with serum AST ($p = 0.04$) and ALT ($p = 0.01$) activities. Notably, among carriers of the *PNPLA3* p.148M allele, presence of the *HSD17B13* allele was associated with lower AST ($p = 0.006$) and ALT ($p = 0.002$) activities, underscoring the protective effects of this variant. Finally, the *PNPLA3* p.I148M polymorphism was associated with an increased risk of presenting with liver stiffness ≥9.2 kPa (common OR = 1.50, $P = 0.03$), i.e. with significant fibrosis (Caballería et al. *Clin Gastroenterol Hepatol* 2018*)* and this association remained significant ($p = 0.04$) in a multivariate model including the *HSD17B13* polymorphism.

Conclusion: Previous genetic studies in NAFLD patients were mostly performed in tertiary academic referral centres. Here, by analysing patients from a "real life" NAFLD cohort, we further underscore the role of the *PNPLA3* variant as the central genetic trigger and modulator of NAFLD. We also demonstrate that the *HSD17B13* polymorphism can attenuate some of the harmful *PNPLA3*-associated effects.

P03-13YI Urea cycle enzymes dysregulation is linked to a more agressive NAFLD phenotype

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Background and aims: The main aim of this study was to evaluate changes in the ureagenesis cycle in patients suffering from different stages of NAFLD.

Method: 17 biopsy-proven NAFLD patients were included for IHC and gene expression analyses. Patients were selected as bland steatosis (n = 10) defined as steatosis presence, nor steatohepatitis or fibrosis and NASH with fibrosis (n = 7). Total RNA from frozen liver biopsies was isolated. qRT-PCR reactions were carried out in duplicates using 7500 Fast Real-Time PCR System. RNA normalization was performed by amplification of RNA 18S as an endogenous control. The $2^{-\Delta\Delta CT}$ method was used for the analysis of the relative gene expression and results were expressed as fold change. For IHC evaluation, by using standard procedures, liver sections were incubated with GS, OTC1 and CPS1 antibodies. IHC quantification was performed by using the IHC Profiler plugin. Finally, 383 biopsy-proven NAFLD patients were genotyped for rs1047891 variant in carbamoyl phosphate synthetase-1 (CPS-1).

Results: Transcriptomic activity of these regulatory enzymes involved in urea synthesis revealed a significant downregulation in bland steatosis in comparison with healthy controls (HC): carbamoyl phosphate synthetase-1 (CPS-1) was 0.3fold [CI95% 0.02-0.34] *vs* HC (1 fold [CI95%1.32-0.75]) (p = 0.0003), but not statistically significant in NASH-fibrosis patients (0.5 fold [CI95% 0.02-1.35]) (p = 0.2). Additionally, there was a downregulation in ornithine transcarbamylase 1 (OTC-1) in bland steatosis (0.28 fold [CI95% 0.05-0.42]) *vs* HC (1 fold [CI95% 1.18-0.84]) (p < 0.0001), as well as in NASH-fibrosis patients when compared to HC (0.50 fold [CI95% 0.06-1.19]) (p = 0.01). GS protein expression was found to be diminished in NASH-fibrosis patients (p = 0.024). Subsequently, GS was found to be inversely correlated to fibrosis stages (r = -0.522; n = 17; p = 0.032) and globally, higher NAS Score values (r = -0.610; n = 17, 0.009). Similarly, CPS1 was found to be downregulated in NASH-fibrosis patients when compared to steatosis simple (p = 0.004), and the correlation with fibrosis was also found to be significant (r = -0.736; n = 12; p = 0.006), as well as with ballooning (r = -0.666; n = 12; p = 0.018) and steatosis degree (r = -0.562, n = 12; p = 0.037). After univariate and multivariate analyses, A-alelle from rs1047891 from CPS1 was found to be protective against liver fibrosis onset (O.R. 0.62 (CI95% 0.39-0.99; p = 0.047).

Conclusion: NASH and significant fibrosis are associated with a reduction in both gene and protein expression pattern of urea cycle enzymes. Further, A-allele from a functional variant on CPS1 may protect from fibrosis susceptibility.

Figure: IHC findings in bland steatosis and NASH-fibrosis patients.

	Bland Steatosis	NASH-Fibrosis
GS		
OTC1		
CPS1		
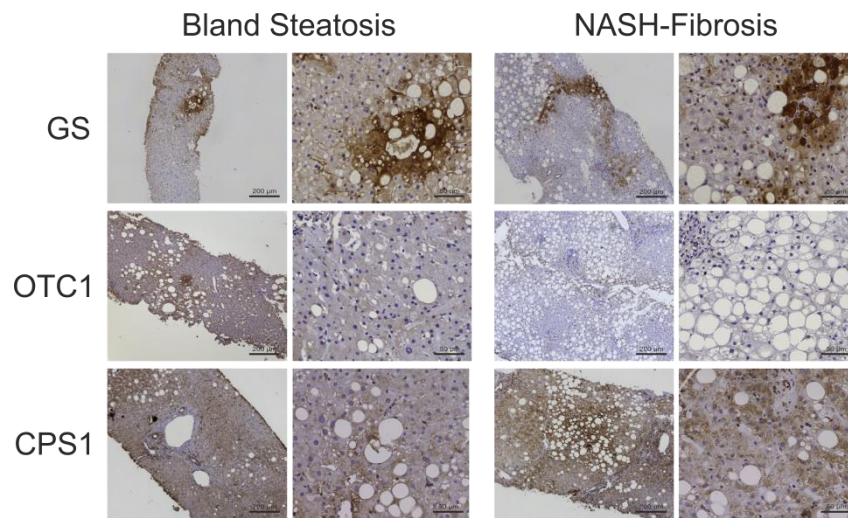
P03-14YI The features of gut microbiota composition in patients with NAFLD

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver disease worldwide. The gut microbiome produces host-derived parts to produce metabolites, which have a wide array of effects on host metabolism and immunity. 'Dysbiosis' of the gut microbiome, commonly considered as perturbation of microbiome diversity and composition, has been associated with intestinal and extra-intestinal diseases, including NAFLD. The aim of this study was to analyse the gut microbiota composition in patients with NAFLD an its connection with liver biochemical markers.

Method: 43 patients with NAFLD with average age 46.97 ± 2.53. and BMI 27.43 ± 0.74 were examined in "Medicover Ukraine". Biochemical evaluation included lipid profile, C-reactive protein, ALT, AST, GGTP, CRP, bilirubin (total, direct, indirect), apolipoprotein B, apolipoprotein A1. Determination of microbial composition at the level of major microbial phyla was carried out by identification of total bacterial DNA, and DNA of Bacteroidetes, Firmicutes and Actinobacteria was performed with quantitative real-time PCR (qRT-PCR), using gene-targeted primers. Ultrasound examination was proved to all patients. The criteria for fatty infiltration existence was a diffuse increase in the echogenicity of the liver parenchyma, decreased attenuation on the liver and ratio between the brightness level of the liver and the right kidney that was calculated for the hepato-renal index (HRI) determination.

Results: The percent composition of microbiota included next proportions of bacteria-Bacteroidetes-16.7 ± 2.99, Firmicutes-45.3 ± 2.99, Actinobacteria-25.9 ± 1.9, Firmicutes/Bacteroidetes ratio (F/B)-6.47 ± 1.55.). Strong negative correlation between Bacteroidetes and Firmicutes (r = -0.93), Bacteroidetes and Firmicutes/Bacteroidetes index (r = -0.65) and Bacteroidetes and Actinobacteria (r = -0.89) was marked. Moreover, there was strong positive correlation among F/B index and triglycerides (r = 0.42) and ALT (r = 0.4). Surprisingly, there was no correlational relationship between BMI and any of bacteria phyla.

Conclusion: The Firmicutes increasing leads to ALT and TG level growth, that is the factor of NASH risk. Simultaneously, the Firmicutes decreasing are strongly connected with Actinobacteria amount reduction, with Bacteroidetes increasing. Thus, Bacteroidetes could be the preventing factor of NAFLD progression, while the F/B index is potential marker of NASH progression in patients with NAFLD.

Figure: Figure 1. The correlational relationship between gut microbiota composition and biochemical markers in patients with NAFLD.
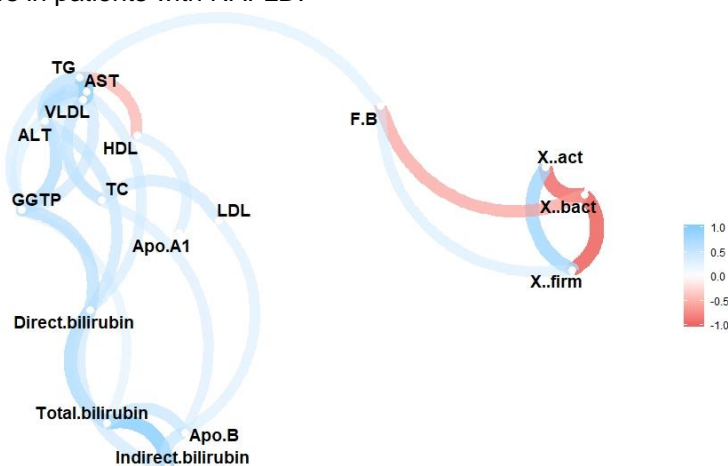
P03-15YI Nuclear NFATc1 regulates pro-apoptotic ER stress signaling protein CHOP, and progresses NAFLD to NASH

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver diseases worldwide having 20%-30% prevalence. It initiates with lipid deposition in hepatocytes (steatosis) and progress to non-alcoholic steatohepatitis (NASH) and Hepatocellular carcinoma. Numerous studies documented aberrant NFAT signalling inducing inflammation and tumor development in other organs e.g. pancreas. This study intends to investigate NFATc1 mediated signalling and its regulated genes in NAFLD-NASH progression.

Method: Immunohistochemistry (IHC) analysis of NFATc1 activation was performed in human NAFLD patient biopsies. NFATc1 expression levels in mouse primary hepatocytes and AML12 cells was confirmed after palmitate treatment by western blot and immunofluorescence (IF). 8 weeks old C57BL/6 mice with hepatocyte specific constitutively active, knock-out and wild type NFATc1 expression were fed with normal and western diet for 20 weeks, respectively. Steatosis, inflammation and fibrosis were analyzed in mouse liver tissues using Hematoxylin eosin staining, IHC and picrosirius-red staining. Whole transcriptome from AML12 cells after transfection with constitutively active NFATc1 construct was analyzed and results were validated in AML12 cells and mouse liver tissue lysates with RT-PCR and western blot. Cytokine proteome profiling was performed in mouse liver tissue lysates.

Results: NFATc1 is activated in hepatocytes following treatment with western diet/Palmitate both *in-vivo* and *in-vitro*. Aberrant expression of NFATc1 induced progressive hepatic inflammation and accelerates deposition of extracellular-matrix in NFATc1*WT* and NFATc1*+/+* mouse model. NFATc1 *knock-out* protected mice against western diet induced inflammation and fibrosis. RNA-seq analysis in AML12 cells transfected with constitutively active NFATc1 showed upregulation of pro-inflammatory and pro-apoptotic ER stress signaling in particular interleukin-1 and PERK regulated genes signaling. PERK regulated C/EBP homologous protein (CHOP) induction leads to caspase-1 mediated apoptosis and induction of pro-inflammatory cytokines, IL1α and IL1β in hepatocytes. While NFATc1 silencing protects CHOP induction and downstream apoptosis as analyzed by TUNEL assay and western blot. Similar effects were found in mice pretreated with western diet. We also found NFATc1 dependent changes in inflammatory cytokines *in-vitro* and *in-vivo* upon western diet treatment. Finally, we observed NFATc1 dependent NAFLD progression to NASH.

Conclusion: Together, our ongoing study suggests a role for NFATc1 in liver damage and proposes a model in which NFATc1 induction drives inflammation and fibrosis presumably via inducing hepatocytes ER stress induced apoptosis and pro-inflammatory cytokines.

Figure:
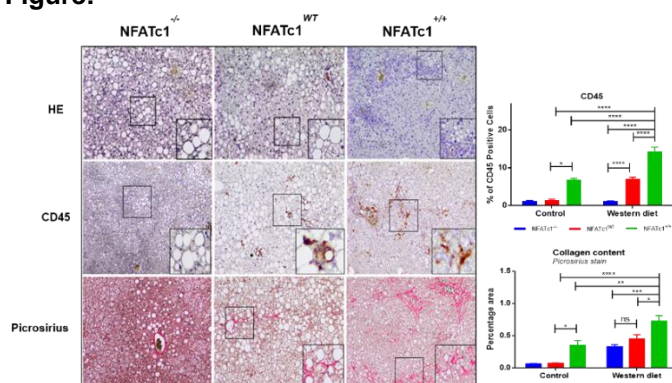
P03-16
European NAFLD policy review
<u>Jeffrey Lazarus</u>[1], **Mattias Ekstedt**[2], **Giulio Marchesini Reggiani**[3], **Jillian Mullen**[4], **Katja Novak**[5], **Juan M Pericàs**[1 6], **Elena Roel**[1 7], **Manuel Romero Gomez**[8], **Vlad Ratziu**[9], **Frank Tacke**[10], **Helena Cortez-Pinto**[11], **Quentin Anstee**[12 13]
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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a growing public health problem worldwide and has become an important field of biomedical inquiry. We aimed to determine whether European countries have mounted an adequate public health response to NAFLD and non-alcoholic steatohepatitis (NASH).
Method: In 2018 and 2019, NAFLD experts in 29 European countries completed an English-language survey on policies, guidelines, awareness, monitoring, diagnosis and clinical assessment in their country. The data were compiled, quality checked against existing official documents and reported descriptively.
Results: None of the 29 participating countries had written strategies for NAFLD. Two countries (7%) had mentions of NAFLD or NASH in related existing strategies (obesity and/or alcohol) (see Figure). Ten (34%) reported having national clinical guidelines specifically addressing NAFLD. Eleven countries (38%) recommended screening for NAFLD in all patients with either diabetes, obesity and/or metabolic syndrome; while 15 (52%) countries recommended screening for these conditions among NAFLD patients as well as other conditions. Five countries (17%) had referral algorithms for follow-up and specialist referral in primary care, and 7 (24%) reported structured lifestyle programmes aimed at NAFLD. Seven (24%) had funded awareness campaigns that specifically included prevention of liver disease. Three countries (10%) reported having civil society groups focus on NAFLD and three countries (10%) had national registries that include NAFLD.
Conclusion: We found that a comprehensive public health response to NAFLD is lacking in the surveyed European countries. This includes policy in the form of a strategy, clinical guidelines, education, awareness campaigns, civil society involvement, and health system organization, including registries.
Figure:

Figure. Health strategies/action plans in the surveyed 29 European countries, and mentioning of NAFLD or NASH in them.
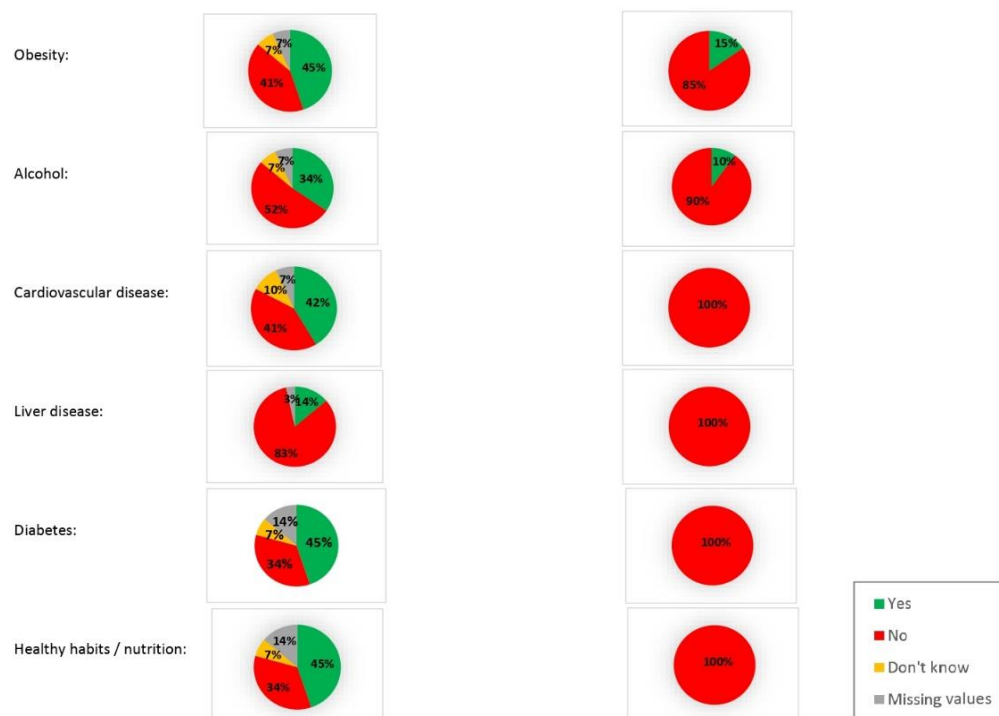
P03-18YI Differential therapeutic effects of single and pan-PPAR agonists on experimental steatohepatitis and hepatic macrophage biology

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Background and aims: Peroxisome proliferator-activated receptors (PPARs) are essential regulators of glucose and lipid metabolism in the liver and adipose tissue. Furthermore, they are also expressed in immune cells, notably macrophages, where they act as modulators of inflammation and fibrogenesis. We aimed to compare selective PPARα, γ and δ agonists with the pan-PPAR agonist lanifibranor in a therapeutic setting and investigate the effect on hepatic macrophages.

Methods:
Male C57BL/6J mice were fed a choline-deficient, amino acid-defined high-fat diet (CDAA-HFD) for 8 or 12 weeks. After 6 weeks of diet, mice were treated via oral gavage with lanifibranor (30mg/kg/day) or selective PPARα (fenofibrate, 100mg/kg/day), γ (pioglitazone, 30mg/kg/day) and δ (GW501516, 10mg/kg/day) agonists for 2 (short-term) or 6 (long-term) weeks. Acute liver injury was induced by carbon tetrachloride (CCl_4). Bone marrow-derived macrophages were stimulated *in vitro* with palmitic acid and treated with the PPAR agonists.

Results: Lanifibranor significantly improved all histological features of steatohepatitis, including liver fibrosis, and reduced the hepatic triglyceride and hydroxyproline content. Fenofibrate improved liver histology, especially steatosis and fibrosis, to a lesser extent, whereas pioglitazone and GW501516 had minor effects. Infiltrating hepatic monocyte-derived macrophages (MoMF) and monocytes were reduced following treatment, especially with lanifibranor. Liver and blood lymphocyte populations were unaffected. Short-term lanifibranor treatment already attenuated steatosis, inflammation and even liver fibrosis, and starkly reduced the number of MoMF. However, PPAR agonists did not directly inhibit monocyte migration, because hepatic MoMF recruitment was not altered after acute CCl_4 injury. *In vitro* macrophage stimulation with palmitic acid induced the expression of pro-inflammatory and lipid metabolism genes. Lanifibranor treatment uncoupled these pathways, as lipid metabolic genes were upregulated and inflammation dampened.

Conclusion: Pan-PPAR agonists combine the beneficial effects of selective PPAR agonists and may counteract inflammation and disease progression more potently. Macrophage infiltration is reduced indirectly, and pro-inflammatory activation is attenuated by lanifibranor, which might contribute to its therapeutic effects.

P03-19 GLI-3 mutation influences weight gain, glucose tolerance and hepatic innate immune populations in a model of non-alcoholic fatty liver disease

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Background: Non-alcoholic fatty liver disease (NAFLD) now the commonest liver disease, ranges through steatosis, non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and hepatocellular cancer. Interestingly, monocytes infiltrate injured livers in NASH where they potentially have dual roles, perpetuating inflammation or promoting resolution of inflammation and fibrosis. Intrahepatic monocyte infiltration of the damaged liver represents an important component of the innate immune response in NAFLD. When the liver is under pathological stress, as in NASH, Kupffer cells (liver macrophages) a key component of the liver's innate immune response can be differentiated from infiltrating bone marrow-derived mononuclear cells. **Aims:** Investigate the involvement of the hedgehog signalling pathway (Hh) in the innate immune response in NASH using Gli3± mice as a model of Hh activation with wild-type (WT) controls.

Method: WT and Gli3± male mice were fed for 20weeks an obesogenic NASH-inducing diet, high fat-high sugar diet (HFD) as we've previously shown. Body weights were recorded weekly. After 20 weeks, mice were fasted overnight and subjected to an intra-peritoneal glucose tolerance test (IPGTT), to detect the presence of insulin resistance. Subsequently, the mice were culled, the livers harvested, weighed, digested and analyzed by flow cytometry to determine the composition of their innate immune cell populations. Total body mass and white adipose tissue mass were also assayed.

Results: Over 20 weeks, the WT Group gained more weight than the Gli3± group and did so along a steeper-faster trajectory than the Gli3± mice. The final weights of the WT was also higher than the Gli3± Group. However, with IPGTT, the Gli3± group had a higher peak at 15 minutes compared to WT and the area under the curve was also higher with the Gli3± group, indicating impaired glucose tolerance and insulin resistance. The Gli3± group moreover had a lower liver: body mass ratio compared to the WT group, implying a smaller liver volume. Whilst there was no statistically significant difference in the white adipose tissue (WAT) mass between WT and Gli3± group, the Gli3± appeared to have a larger WAT mass. Flow cytometric analyses of the livers showed an increased proportion of cells that expressed LY6G+ (a marker of monocyte, granulocyte and neutrophil) in the Gli3± mice compared to WT. However, the proportion of macrophages, Ly6C+ cells and NK cells showed no differences between the two groups.

Conclusion: The transcription factor Gli3 is involved in NAFLD. A reduction in Gli3 leads to slower weight gain, a lower liver:body mass ratio, an increase in the hepatic granulocyte/neutrophil populations but with no differences in macrophages or NK cells between the two groups. The Role of Gli3 in NASH needs more investigation.

P03-20YI Effect of treatment with dulaglutide on metabolic function and liver tests in patients with NAFLD and diabetes mellitus type 2

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Background and aims: To date, there is no universally accepted treatment regimen for patients with NAFLD and type 2 diabetes. GLP1- agonist dulaglutide has been approved for treatment diabetes mellitus type 2 (T2DM) and metabolic improvement of NAFLD on GLP-1treatment has been shown on rodent models. Aim of the study is to assess of the effects of dulaglutide on metabolic and liver parameters in patients with diabetes mellitus type 2 and NAFLD

Method: After approval by the local ethics committee, 57 patients with NAFLD with or without T2DM (BMI>27kg/m^2) were included in the study. The T2DM was diagnosed based on ADA/EASD criteria at least 6 months before inclusion. All patients undergo physical examination every visit with height, weight, waist and hip circumference measurements, monthly blood sampling tests, ultrasound, transient elastography (TE), liver biopsy at baseline and at the end of study (planned duration 52 weeks). Statistical analysis was made using SPSS v21.0 using parametric (Fisher test) and nonparametric methods (Mann-Whitney, Chi-square test). P values less than 0.05 were considered statistically significant. Results presented in median with interquartile range (IQR). This is an interim result after 12 weeks treatment period.

Results: Median age 56 [47; 63] years with BMI 35, 4 [31, 2; 41] kg/m2. Among these patients 36 (66%) had T2DM. We found statistically significant decrease of HвA1c in T2DM group 7, 5% at baseline vs 6, 4% in 12 weeks of treatment p < 0.005. After 12 weeks treatment body weight loss was 3, 8 % [1, 26; 5, 71] in all patients. ALT, GGT and triglycerides level were significantly lower after 12 weeks treatment with dulaglutide. There was no effect of dulaglutide on cholesterol level. No serious adverse event (AE) was register during this period. The most common AE was gastrointestinal. There were no episodes of hypoglycemia in the group of non-diabetic patients.

Conclusion: Patients with NAFLD may benefit from treatment with dulaglutide due to weight loss and liver tests impairment. Further studies are needed to investigate the role of GLP-1 agonist treatment on inflammation and liver fibrosis progression in patients with NAFLD.

Figure:

	T2DM+NASH			nonT2DM+NASH		
	Baseline	**Week 12**	**p**	**Baseline**	**Week 12**	**p**
BMI, kg/м^2, Me (IQR)	34,3 (31,1; 41)	32,9 (30,8; 40,7)	**<0,001**	36,8 (31,5; 39,1)	35,7 (31,5; 37,5)	**0,003**
Weight, kg, Me (IQR)	102 (85,5;112,5)	96,9 (82,1; 106,7)	**<0,001**	104,5 (89,2; 114,5)	102,7 (86; 111,1)	**0,002**
HвA1c, %, Me (IQR)	7,5 (6,1; 8,9)	6,4 (5,7; 6,9)	**0,008**	5,5 (5,2; 6,7)	5,3 (5,2; 5,7)	**0,007**
ALT, IU/ml Me (IQR)	70,7 (36,3; 102,2)	49,4 (30,1; 91,3)	**0,03**	49,5 (23,8; 83,5)	37,7 (22,5; 56,9)	**0,002**
AST, IU/ml Me (IQR)	45,6 (30,5; 74,3)	35,3 (26,5; 53,1)	**0,254**	31,2 (19,7; 57,4)	23,3 (20,3; 39,9)	**0,025**
Cholesterol, mmol/l, Me (IQR)	4,9 (4; 5,5)	4,8 (3,8; 5,5)	0,885	4,7 (3,8; 5,7)	5 (3,5; 5,4)	0,054
Triglyceride, mmol/l Me (IQR)	1,6 (1,1; 2,3)	1,5 (1; 2,2)	0,19	1,6 (1,5; 2,2)	1,4 (1;1,5)	**0,046**
GGT, IU/ml Me (IQR)	64,3 (35,5; 116,6)	47,4 (32,8; 73,3)	**0,023**	48,1 (31,8; 74,9)	37,9 (31,7; 60)	**0,041**

Effect of metformin therapy on the clinical and functional state of the liver in patients with metabolic syndrome associated with non-alcoholic fatty liver disease

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Background and aims: Evaluate the effect of metformin therapy on the clinical and functional state of the liver in patients with metabolic syndrome associated with non-alcoholic fatty liver disease.
Method: The study included 10 patients with metabolic syndrome associated with non-alcoholic fatty liver disease, aged 42 to 56 years. The examination included anamnesis, physical examination with control of body mass index, clinical and biochemical analysis of blood, determination of insulin resistance, data of ultrasound examination of the abdominal cavity organs. Diagnosis of metabolic syndrome was carried out according to the criteria of NCEP ATP III. The course of therapy with metformin was 16 weeks at a dose of 500 mg 3 times a day.
Results: During therapy with metformin, 7 (70%) patients showed a significant decrease in insulin resistance. Normalization of serum transaminase levels was observed in 6 (60%) patients, a tendency to decrease in indicators-in 4 (40%). Also, in 8 (80%) patients the dynamics of weight loss was noted (by 2-4 kg).
Conclusion: Metformin therapy has a positive effect on the clinical and functional state of the liver in patients with metabolic syndrome.

P03-22 High cholesterol diet and high saturated fatty acid diet: Which is worse for the liver

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Background and aims: High fat diet, mixture of saturated and unsaturated fatty acids, has been often used for making NAFLD model mice, and the addition of cholesterol to it causes more severe form of NAFLD (NASH). However, it is unclear how dietary cholesterol or saturated fatty acid directly or indirectly affects the liver. Therefore, we investigated impacts of short-term feed with high cholesterol diet (HCD) or high saturated fatty acid diet (HSD) on the liver and microbiota.

Method: C57BL/6J mice (n = 15) were divided into 3 groups fed with normal diet (ND); HCD containing cholesterol of 1.5%; and HSD containing 14% (w/w) hydrogenated coconut oil for 4 weeks. The expression of lipid and bile acid metabolism-related genes in the liver and the ileum were analyzed by RT-PCR. Microbiota analysis was performed by T-RFLP analysis. Serum bile acids and hepatic NAD-related metabolites were measured by LC/MS.

Results: HCD and HSD did not affect body weight, but HCD increased liver weight compared to ND and HSD accompany elevated serum ALT levels. The serum levels of cholesterol were increased by HCD and HSD, but that of triglyceride was increased only by HSD. HCD but not HSD suppressed the gene expression of *HMG-CoAR*, *LDLR* and *FAS*, but enhanced that of *ABCG5* and *CD36*. In the same manner, only HCD inhibited the expression of *CYP7A*, and increased that of *BSEP* and *FGFR4* accompanying increased TCA, TCDCA, beta-MCA, and TDCA. Furthermore, HCD decreased the hepatic NAD content with the increased expression of *NAMPT* and *Sirtuin 1*. Finally, HCD significantly reduced Bacteroidetes, but oppositely increased Proteobacteria and Verrucomicrobia at phylum level. In contrast, HSD reduced Bacteroidetes, but increased Actinobacteria.

Conclusion: HCD but not HSD strongly affected liver function and lipid and bile acid metabolism, and HCD also changed the hepatic involvement of the NAD-Sirtuin pathways. HCD and HFD respectively changed microbiota. These results suggest a fine-tuned differential pathway of cholesterol and saturated fatty acid in the liver, and that cholesterol might be more harmful.

Figure: Nil

P04-01YI A multidisciplinary approach to non-alcoholic fatty liver disease (NAFLD) improves cardiovascular risk factors

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Background and aims: Cardiovascular (CV) disease is the leading cause of death in unselected patients with NAFLD. Guidelines strongly recommend a cardiovascular and metabolic assessment because of the strong association between NAFLD and hypertension (HT), dyslipidaemia and type 2 diabetes (T2DM). The need of a multidisciplinary (MD) approach is highlighted in many reviews, but there is a lack of clinical data to demonstrate its effectiveness.

The aim of our study was to assess the efficacy of a MD clinic through control of metabolic comorbidities and surrogate markers of liver involvement.

Method: Data collection of 273 patients referred to a MD NAFLD clinic, comprehensive of a hepatologic consultation, cardiovascular risk assessment and dietetic counseling. Anthropometric measurements, blood pressure (BP) and blood tests with lipid, hepatic, glycaemic profile were recorded. QRisk-3 was calculated at baseline and at the latest follow-up visit.

Results: Mean age was 56.4 ± 12.1 years, with 57% males. The median follow-up was 18 months (range 1-118). HT was present in 67% of patients, while DM2 and dyslipidaemia were present in 49.8% and 93%, respectively and 13.2% had a positive history of CV events. More than half patients were obese (n = 135, 60.3%) and/or had raised transaminases (n = 165, 61%). At baseline, dyslipidaemia management was suboptimal in 68 patients (26.8%), while 57 (41.9%) patients with T2DM and 36 (19.6%) patients with hypertension needed modification of their treatment. During follow-up, there were statistically significant improvements in ALT ($p = 0.013$), AST ($p = 0.013$), systolic and diastolic BP ($p = 0.002$ and 0.014 respectively), total cholesterol ($p < 0.001$), low density lipoprotein (mean drop from 3.2 to 2.2 mmol/L, $p < 0.001$) and glycated haemoglobin in diabetic patients (mean drop from 70.2 to 62.5 mmol/mol, $p = 0.04$). 142 patients achieved weight loss during the follow-up; of them 19 patients (8.2%) lost ≥10% of the baseline weight, 14 patients (6%) ≥7%, 17 patients (7.3%) ≥5%. The total number of patients with a Qrisk-3 score≥10% decreased from 156 (62.7%) to 97 (48.5%).

Conclusion: A multidisciplinary NAFLD approach was effective in improving liver-related and CV risk factors. The high prevalence of comorbidities and the high percentage of sub-optimally treated patients highlight the need of this approach. A strong collaboration between primary and secondary care is essential to implement and maintain these improvements in the long term.

P04-02YI Risk factors in type 2 diabetic mellitus patients

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Background and aims: Non-alcoholic fatty liver disease is becoming the most common cause of chronic liver disease. The aim of this study was to identify factors associated with significant liver disease in a cohort of type 2 diabetes patients.

Method: Patients were recruited prospectively in the study. Evaluation of liver fibrosis was made using Transient Elastography (FibroScan), performed in fasting conditions. Each patient was evaluated for the presence of viral hepatitis (B and C), and an AUDIT-C score was performed to exclude alcohol abuse. Variables tested for the association with significant liver fibrosis were: age, body mass index (BMI), abdominal circumference, hypertension, years after diagnosis of diabetes, glycemia, statin treatment, oral antidiabetics treatment and insulin treatment. Multivariate regression was used to assess the association between significant liver fibrosis and other variables. The cut-off value used to define significant fibrosis was >8.5 kPa [1].

Results: Out of 641 diabetics evaluated, after the exclusion of those with associated viral hepatitis, with an AUDIT-C score ≥8 and with unreliable LSM, the final analysis included 407 patients (mean age 60.5 ± 9.6, 228 women, 179 men). At least significant fibrosis was found in 28.7% (117/407) patients. In univariate analysis we found a significant correlation with body mass index (BMI), HbA1C, ALT, AST, glycemia and age>60 years. Multivariate regression analysis confirmed the correlation with age [OR = 5.9, $p = 0.009$], BMI [OR = 1.08, $p = 0.0008$], AST [OR = 1.03, $p = 0.04$] and HbA1c [OR = 1.27, $p = 0.001$]

Conclusion: In our group, 28.7% of diabetic patients had at least significant fibrosis. Body mass index (BMI), age and Hb A1c were associated with significant liver fibrosis.

References:

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P04-03 Psoriasis and liver damage in HIV-infected subjects

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Background and aims: Psoriasis (PS) is one of the most frequent dermatological inflammatory disorders in HIV infected subjects. However, NAFLD associated to PS is not well known in this population.
Our aim was to describe the prevalence of NAFLD associated to PS and to analyse what factors predict liver damage in a large cohort of HIV-infected subjects in the HAART (high antiretroviral therapy) era.
Method: Cross-sectional study in a 5452 HIV-infected subjects cohort from a Clinical Unit in Spain. All cases of PS were identified and clinical characteristics analysed. Two dermatologists classified psoriasis and calculated highest historical PASI index for every subject; we defined severe psoriasis (sPS) as PASI index >10 and/or psoriatic arthritis. We studied the clinical records to non-invasively identify liver steatosis (liver ultrasound, Controlled Attenuation Parameter or TyG/HSI indexes) and liver fibrosis (transient elastography, APRI, FIB-4). Risk factors of liver steatosis and liver fibrosis were studied by logistic regression analysis adjusted by age, BMI, HCV-coinfection and HIV-infection time duration.
Results: Eighty subjects with PS were identified (prevalence of 1.5% (IC 95% 1.1-1.8). The main clinical characteristics are shown in table 1. There were 27, 5% with sPS. In the whole psoriatic population, steatosis was detected in 74, 7% (CI95% 64, 5-84, 9). In the subgroup with sPS, steatosis prevalence was 100%. sPS was an independent risk factor for liver steatosis (OR 14, 9 CI 95% 1, 8-126; p = 0.03).Liver fibrosis ≥F3 was associated with age (OR 1, 08 CI95% 1, 01-1, 2; p = 0.033), HCV infection (OR 4, 6 CI95% 1, 6-13, 2) and sPS (OR 3, 4 CI95% 1, 1-10, 8). We did not find association with HAART, HIV-infection stage nor metabolic conditions.
Conclusion: HIV-infected subjects have similar prevalence of PS to general population. Liver steatosis is highly prevalent in this population. Those HIV-infected subjects with severe psoriasis have a higher risk of liver steatosis and significant fibrosis. They deserve specific evaluation and follow-up.

Figure:

Characteristics	Non-severe-Psoriasis N = 58	Severe-psoriasis N = 22
Age*, years	50	52
Female gender (%)	24	13.6
C AIDS stage (%)	9	18
HCV-coinfection (%)	31	50
IDU-transmission (%) ç	28	55
HIV-infection controlled (%)	98	88
Nadir CD4+*, cel/µL ç	230 (160-325)	124 (70-194)
Metabolic syndrome (%) ç	21 (9.4-32.0)	45 (22.4-68.5)
BMI*	24 (22-27)	26 (23-27)
Glucose*, mg/dL ç	92 (85-101)	101 (91-117)
Triglycerides*, mg/dL ç	103 (77-160)	135 (115-179)

*Median (p25-p75) Ç p < 0.05

P04-04YI Predictive circulating hormone biomarkers for NAFLD patient stratification

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__Background and aims:__ Non-alcoholic fatty liver disease (NAFLD) is the most common cause of liver disease in the Western world. The disease spectrum ranges from benign hepatic steatosis (NAFL) to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and ultimately hepatocellular carcinoma. Circulating biomarkers may circumvent the need for invasive diagnostic procedures like liver biopsy. Here, we assessed the potential of circulating hormones as NAFLD non-invasive biomarkers

__Method:__ Using specific enzyme-linked immunosorbent assays, serum levels of adipocytokines, adiponectin and leptin, as well as insulin-like growth factor-1 and -2 (IGF-1, IGF-2) were evaluated in 2 cohorts of morbidly obese patients, with clinical and biopsy proven diagnosis of NAFLD (cohort A: n = 145; cohort B: n = 59). Sera of lean and obese disease-free individuals were also analyzed in both cohorts (cohort A: n = 32; cohort B: n = 20). Hormone levels were correlated with histology findings and clinical parameters. Cohort A was used to build a statistical model, which was then validated in cohort B.

__Results:__ In both cohorts, leptin levels were significantly increased in patients with NAFL and NASH when compared to healthy controls ($p <0.0001$), with area under the receiver-operating characteristic (AUROC) of ~ 0.9 ($p <0.05$). Circulating levels were similar between lean and obese controls, suggesting that obesity is not a confounding factor. Results showed also that adiponectin circulating levels were significantly lower in NASH patients comparing to NAFL patients ($p <0.05$ in cohort A, and $p <0.0001$ in cohort B). Further adiponectin showed an inverse correlation with serum alanine aminotransferase and triglycerides ($p <0.01$), supporting a role for this hormone in NAFLD pathophysiology. Strikingly, in cohort A, IGF-1 levels were significantly lower in patients with fibrosis stage F0-F2, comparing with F3-F4 patients ($p <0.005$), with an AUROC value of 0.71 ($p <0.01$). In this cohort, IGF-2 did not retrieve any significant differences.

__Conclusion:__ Adiponectin, leptin and IGF-1 are potentially valuable tools for non-invasive stratification of NAFLD patients. Leptin might discriminate the presence of NAFLD, whereas adiponectin may stratify these patients between NAFL and NASH. In turn, IGF-1 could be a biomarker of advanced fibrosis. Further studies should analyze these hormones in a lean NAFLD control group and assess the impact of other confounding factors.

P04-05 Relationship of semiquantitative scoring systems with computed-assisted digital image analysis for quantification of histological features in NAFLD

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Background and aims: Liver biopsy is frequently used for the assessment of liver disease severity in patients with non-alcoholic fatty liver disease (NAFLD). Interpretation of biopsies is based on semiquantitative scoring systems, carrying a risk of misclassification. In the present study, we evaluate the relationship between biopsy grading scores and the corresponding amount of collagen, fat, inflammation and iron quantified by computer-assisted digital image analysis (DIA).

Method: Prospective, multicenter, comparative study of digital pathology for the quantification of histologic features in liver biopsies of subjects with suspected-NAFLD. Samples were stained with: Picrosirius red for fibrosis detection, Perl's stain for iron, immunohistochemistry with adipophilin for fat and CD45 for inflammation. Grading scores included: METAVIR for fibrosis, Non-alcoholic Steatohepatitis Clinical Research Network (NASH-CRN) for steatosis and inflammation, and Deugnier for iron deposits. Stained tissue sections were scanned (Ventana iScan HT®) for DIA. Quantitative variables were expressed as proportional areas (%) of collagen (CPA); fat (FPA); inflammation (IPA); and iron (FePA). Statistical analysis was performed using ANOVA test for comparisons of DIA data among corresponding semiquantitative groups (METAVIR, NASH-CRN); and Spearman's co-efficient for correlation of quantitative data (Deugnier).

Results: The study included 80 patients (58% women; median age, 55 years; range, 48-63) with a median body mass index of 29 (range, 24-32). Median length and portal tracts of biopsies were 21mm (range, 17-24) and 10 (range, 7-13) respectively. CPA values increased significantly with fibrosis stages (7.4 ± 1.9, 8.7 ± 1.3, 8.8 ± 1.3, 10.1 ± 2.4 and 13.5 ± 2.3, F0-F4 respectively, $p < 0.001$). FPA values increased significantly with steatosis stages (3.5 ± 2.2, 9.1 ± 5.0, 13.0 ± 4.2, and 20.3 ± 9.4, S0-S3 respectively, $p < 0.001$). IPA values were significantly different between inflammation stages (5.1 ± 1.7, 6.2 ± 1.8, 7.2 ± 3.3, and 9.9 ± 4.3, I0-I3 respectively $p = 0, 001$). FePA correlated significantly with Deugnier´s score ($r = 0.84$; $p < 0.001$). Results are represented in Figure 1.

Conclusion: Computational DIA provides an accurate evaluation of histological features in NAFLD, showing a high correlation with current semiquantitative scores. DIA measurements should be now validated towards *in vivo* non-invasive biomarkers.

Figure: Distribution of DIA data across semiquantitative histologic scores.
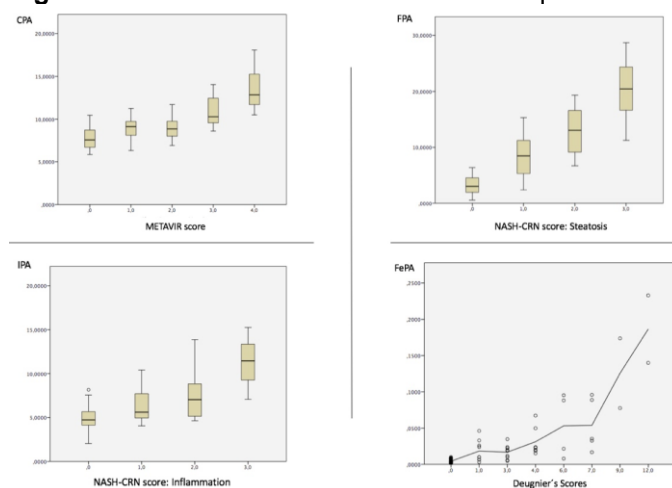
P04-06YI The role of adipocyte-derived extracellular vesicles in the development of NAFLD

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Background and aims: Recent studies have produced evidence that extracellular vesicles (EV) released from adipocytes and adipose tissue (AT) could have important roles in inter-organ communication leading to ectopic fat accumulation in the liver (*I.e.*, the development of non-alcoholic fatty liver disease, NAFLD). However, despite the promising results based on the analyses of the contents of AT-derived EV, the predicted EV-mediated effects in the liver have been rarely confirmed *in vitro* or *in vivo*. In our experiments, we aim to provide the first, detailed mechanistic data about EV-mediated crosstalk between AT and the liver *in vitro.* To examine this, adipocyte-EV mediated changes in hepatocyte fatty acid metabolism and signaling inducing insulin resistance, inflammation and fat accumulation were studied thoroughly. By doing this, the mechanisms by which the crosstalk between AT and the liver may promote pathological hepatic events highly relevant to NAFLD were investigated.

Method: In our experiments, we isolated EV from human Simpson Golabi Behmel Syndrome (SGBS) adipocyte cell line, and transferred EV to immortalized human hepatocyte (IHH) cultures. EV were isolated by differential ultracentrifugation, and the number of secreted EV as well as their size distribution was analyzed by Nanoparticle tracking analysis (NTA). The effects of EV on IHH fatty acid metabolism and signaling promoting insulin resistance, inflammation and fat accumulation were studied by qPCR. The amount of fatty acids were analyzed by the fluorescent labeling of intracellular lipids and confocal microscopy. In addition, the fatty acid profiles of hepatocytes and EV were determined by mass spectrometry-gas chromatography.

Results: NTA analysis revealed that fat-laden, mature SGBS adipocytes secrete a substantial amount of EV. Size distribution analysis obtained by NTA further unveiled that the size of EV secreted was rather small, the average diameter being 130 nm, indicating that most of EV might have been exosomes and microvesicles.

Conclusion: Our results suggest that EV are important communicators between AT and the liver, which potentially has a role in fat accumulation and development of NAFLD. In addition, EV will be isolated from visceral and subcutaneous AT of bariatric surgery patients, as well as from primary adipocytes of patient AT samples. Thus, we will provide reliable and detailed information about EV-mediated crosstalk between AT and the liver.

P04-07 PNPLA3 gene polymorphism (rs738409) and non-alcoholic fatty liver disease risk in women with polycystic ovary syndrome

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Background and aims: The patatin-like phospholipase 3 gene polymorphism (PNPLA3, rs738409[G], encoding I148M) is one of the most important genetic determinants of non-alcoholic fatty liver disease (NAFLD) and has been consistently associated with increased hepatic fat levels and histological severity on different populations. Increasing evidence demonstrates the association of polycystic ovary syndrome (PCOS) and NAFLD, both associated with obesity, insulin resistance and metabolic syndrome. The aim of this study was to describe the prevalence of PNPLA3 gene polymorphism in women with PCOS, in addition to its association with hepatic steatosis, fibrosis and metabolic disorders.
Method: PCOS patients were enrolled at reference services of NAFLD and PCOS. All the patients were analyzed for the presence of the PNPLA3 gene polymorphism, hepatic steatosis at ultrasound, liver enzyme abnormalities and disorders of glycemic metabolism. In patients with steatosis, transient hepatic elastography (Fibroscan®, Echosens) was performed to assess liver stiffness. Another causes of hepatopathies were excluded.
Results: This study included 155 patients aged between 16 and 56 years. The prevalence of NAFLD was 72.9% and fibrosis was present in 45.1% of these patients. The genotypic frequencies of the polymorphism were 57.5% for heterozygous (C/G), and 8.3% for homozygous (G/G). A higher prevalence of the PNPLA3 gene polymorphism was observed between patients with NAFLD, with 45.1% heterozygous (C/G) and 9.7% homozygous (G/G), with a trend to statistically significant difference with the group without NAFLD (p = 0.0725). Half (50.9%) of patients with fibrosis have heterozygous form (C/G) of PNPLA3 gene polymorphism, and 0.58% presented with the homozygous form (G/G), with no statistically significant difference with group without fibrosis (p = 0.7033). In the univariate analysis there was no difference on the prevalence of PNPLA3 gene polymorphism for the hyperandrogenic and normoandrogenic PCOS phenotypes. Patients with glucose intolerance presented higher prevalence of G allele (66, 6%), with a trend to statistically significant difference in comparison with normoglycemic group (p = 0.0889). No significant difference was observed on the presence of G allele between groups with and without diabetes and insulin resistance (respectively, p = 0.6252 and p = 0.1360).
Conclusion: Although the PNPLA3 gene polymorphism (rs738409) is the most important genetic determinant of NAFLD and fibrosis, it seems that association of additional factors on the PCOS patients are more decisive on the development of hepatic steatosis and its advanced forms. The presence of G allele does not confer additional risk for glycemic disorders on PCOS patients.

P04-08YI Metagenomics and molecular phenomics of obesity and hepatic steatosis

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Background and aims: The gut microbiome has been put forward as augmenting factor in development of obesity and hepatic steatosis (NAFLD). We here investigated the discriminatory power of the fecal metagenome to accurately predict obesity and presence of NAFLD or a healthy liver. Next, we investigated, whether the fecal metagenome can explain the variance in obesity-related phenotypes. In addition, we combined fecal metagenomics and plasma metabolomics to reveal metabolic phenotypes linked to NAFLD.

Methods: Fecal metagenome of subjects (n = 177) with a wide variety in BMI was analyzed using shotgun sequencing. Liver biopsies (n = 78) collected during bariatric surgery were scored for NAFLD by expert pathologists. Corresponding (n = 78) metabolomics analyses were performed on fasting- and 2h-post mixed meal test (MMT) derived plasma.

Results: Subjects without (n = 82) and with obesity (n = 95) could be sharply separated based on fecal metagenomics (accuracy of 89%). In addition, gut microbial pathways including amino acid synthesis including histidine was highly enriched in subjects with obesity, whereas degradation of histidine was depleted compared to subjects without obesity. Regression analysis revealed that 45, 5% and 60% of the variance in BMI can be predicted by the gut microbiome and gut microbial metabolic pathways, respectively. The variance in TG and HDL could be explained by 6% and 9%, respectively, whereas the variance in LDL, HbA1c and fasting glucose could not be explained by the gut microbiome. Although fecal metagenome did not discriminate between NAFLD and healthy liver, this separation could be made based on fasting (80% accuracy) and post MMT (75% accuracy) metabolomics data. Of interest, glycoursodeoxycholate and N4-acetylcytidine were highly enriched in subjects with NAFLD vs those with healthy liver.

Conclusion: Fecal metagenome was a strong predictor of obesity and clinically relevant phenotypes thereof. Of interest, the microbiome of subjects with obesity have a higher potential to produce several amino acids compared to subjects without obesity but lacks capacity of catabolizing specific amino acids, such as histidine. In contrast with previous work, a fecal metagenome-based separation between NAFLD or healthy liver was not observed in our cohort of subjects with obesity. Strikingly, however, this separation could be made based on plasma metabolomics data and revealed several interesting metabolites that might be of importance in the development of NAFLD.

P04-09YI Physical activity removes the harmful effects of sedentary behaviours: a prospective look to the Horizon 2020 'Foie Gras' project in Southern Italy

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Background and aims: Consistent physical activity habits (200-300 minutes of moderate physical activity per week, Piercy et al. 2018) reduces the burden for the increasing risk of cardiovascular diseases worldwide. Non-alcoholic fatty liver disease (NAFLD) displays worrying trends accompanying metabolic syndrome and obesity. We investigated the profile of sedentariness and physical activity of a dysmetabolic cohort from Apulia.

Method: Forty-nine outpatients (M:F = 31:18) were assessed for liver steatosis (grade 1-3) by ultrasonographic assessment (Toshiba-Hitachi Noblus, 7.5 MHz probe, Japan) and liver fibrosis (grade F0-F4) by Acoustic Radiation Force Impulse (ARFI, m/s) imaging. A control group of 19 healthy subjects (M:F = 10:9) was used for comparison. The International Physical Activity Questionnaire ('IPAQ', Minetto et al., 2018) was used to profile sitting time (min/week) and physical activity levels (Metabolic Equivalent Tasks, METs/week; 1 MET = 3, 5 ml O_2/kg/min). This pilot study is part of an ongoing lifestyle intervention in NAFLD (NCT03354247).

Results: Liver steatosis and liver fibrosis were more prevalent in obese than in non-obese (1.8 ± 0.1 vs. 0.6 ± 0.1, P < 0.001; 1.5 ± 0.2 vs. 0.7 ± 0.2, P = 0.003; respectively) and in young than adult subjects (1.3 ± 0.1 vs. 0.5 ± 0.2, P = 0.003; 1.2 ± 0.2 vs. 0.5 ± 0.2, P = 0.044; respectively). Patients were heavier than controls (BMI = 32.5 ± 0.8 vs. 22.2 ± 1.4 kg/m2, P < 0.001), but age-matched (46.9 ± 1.6 vs. 41.9 ± 3.7 yrs). Patients sat for less time but tended to do less physical activity than controls (343.9 ± 46.7 and 804.7 ± 184.3 min/week, P = 0.001; 2002.7 ± 468.4 and 9232.7 ± 5992.0 METs/week, P = 0.059; respectively). Obese sat for less time than non-obese subjects (292.5 ± 46.6 vs. 632.8 ± 111.0 min/week respectively, P = 0.009). Sitting time inversely correlated with liver steatosis (r = -0.349, P = 0.004). There were no differences in sitting time and physical activity levels with regards to age (young vs. adult), gender (male vs. female) and liver fibrosis (degree = ≥1 vs. <1).

Conclusion: In a third referral hospital of Southern Italy, liver steatosis was not directly related to sedentary behaviours as measured by sitting time, however it was less prevalent in non-obese and younger subjects. Healthy subjects were more active than their dysmetabolic counterparts, despite displaying higher sedentariness. The 'Foie Gras' project is currently deviating ways to improve adherence to healthy lifestyle habits, including reducing physical inactivity.

Figure:

Figure 1. Prevalence of liver steatosis and liver fibrosis in NAFLD
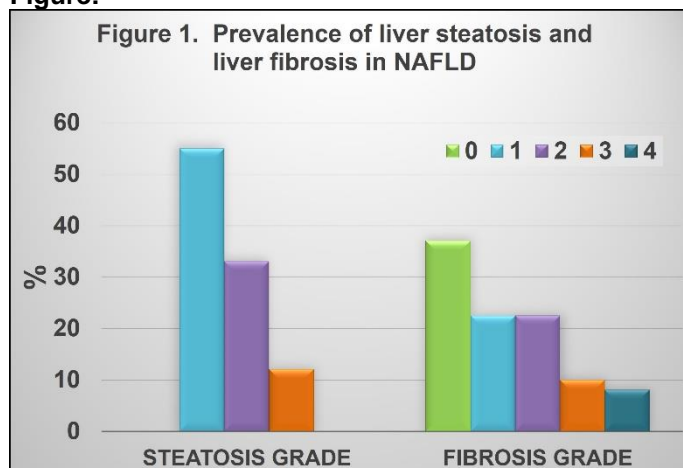
P04-10YI miRNAs as non-invasive biomarkers in Non-alcoholic Fatty Liver Disease (NAFLD)

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Background and aims: To evaluate the differential profile of circulating and exosomal miRNAs in patients with NAFLD and its potential utility as steatohepatitis non-invasive biomarkers.

Method: Thirty-nine patients with biopsy-proven NAFLD were included: 18/39 (46%) with non-alcoholic steatohepatitis (NASH) and 21/39 with simple steatosis (54%). A predesigned array containing the 84 most-common miRNAs in the liver was employed (Qiagen, Valencia, USA). Free miRNAs were isolated from plasma by using miRNeasy serum/plasma kit (Qiagen, Valencia, USA) following manufacturer´s instructions. Further, pull-down of exosomes was performed by using ExoQuickTM from plasma after an overnight incubation, and resuspended into PBS before isolating miRNAs by using miRNeasy miki kit (Qiagen, Valencia, USA). Selected miRNAs candidates were further analysed by qRT-PCR (LightCycler, Roche), employing U6 as the housekeeping gene. Steatohepatitis was defined by overall diagnosis by pathologist, NAS Score and SAF Score. SPSS 24.0 was employed for the statistical analyses.

Results: 62% (24/39) of patients were women, mean age 52 ± 12 and 46% (18/39) suffered from T2DM. In patients with NASH defined by SAF Score, an increase in both circulating miR-224 and miR-200b was observed (fold change 3.4 ± 3.5 and 5.2 ± 6.7; p = 0.011). Further, the same trend was observed in the exosomal profile of miR-200b in NASH vs simple steatosis patients (fold change 11.9 ± 17.1; p = 0.017). NAS Score analysis revealed an increase in circulating miR-224 in NASH (fold change 3.1 ± 3.4; p = 0.027) as well as in exosomal miR-200b (fold change 11.4 ± 17.5; p = 0.05). Finally, steatohepatitis defined by pathologist exhibited an increase in both free and exosomal miR-200b (fold change 3.6 ± 5.4, p = 0.014 and fold change 9.0 ± 5.4; p = 0.021) and exosomal miR-224 (fold change 3.6 ± 4.0; p = 0.038).

Conclusion: Both circulating and exosomal miRNAs were found increased in NASH. Exosomal miR-200b remained significant in all three NASH definition criteria. Further studies are warranted to validate the role of these miRNAs as non-invasive biomarkers.

Figure: Fold change of circulating and exosomal miR-200b and miR-224 in NASH vs steatosis simple defined by pathologist, SAF and NAS score.

Circulating miR-224

Circulating miR-200b

Exosomal miR-224

Exosomal miR-200b
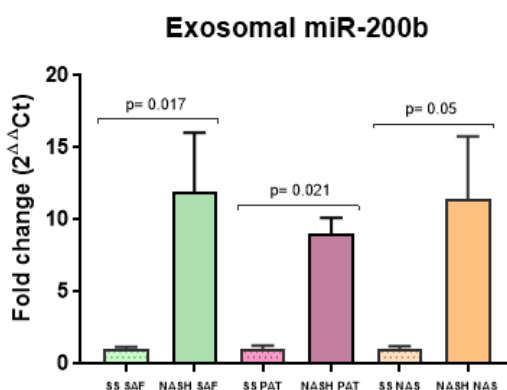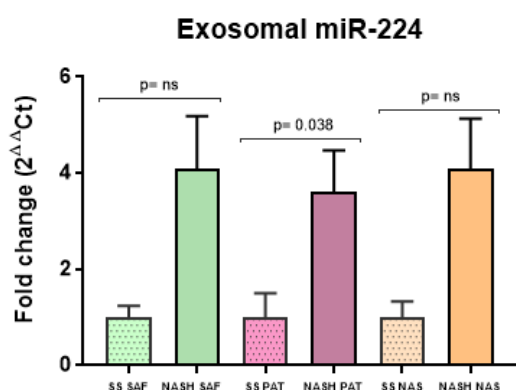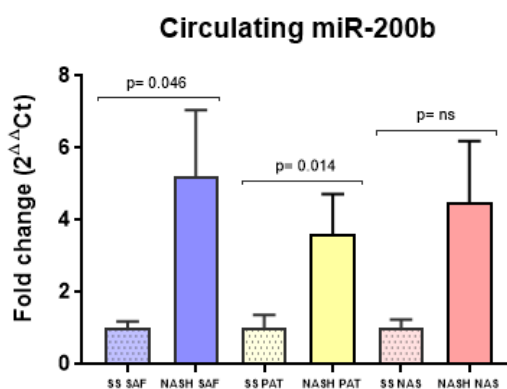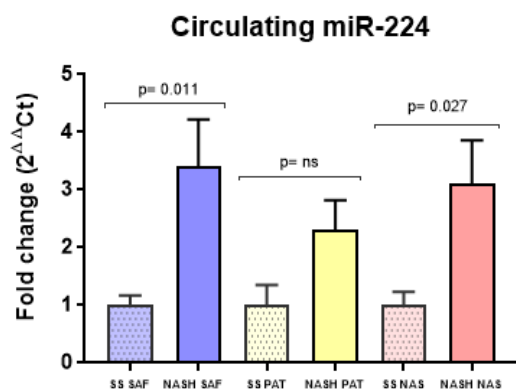
P04-11 Inhibition of alpha 2A adrenergic receptors reduces liver inflammation and fibrosis in experimental NASH

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Background and aims: Fatty liver disease (NAFLD) is increasing worldwide. Patients with the inflammatory state of non-alcoholic steatohepatitis (NASH) have increased risk of developing cirrhosis and hepatocellular carcinoma, whilst fibrogenesis correlates with long-term morbidity and mortality. Norepinephrine (NE), through alpha 2a subtype adrenergic receptors (Adra2aR), may trigger development and progression of NAFLD. NE increases the release of pro-inflammatory cytokines from Kupffer cells (KCs) and we have also shown blocking Adra2a signaling reduces hepatic stellate cell activation. We aimed to study the role of the Adra2aR in a rat model of NASH and investigate Adra2aR antagonism as a possible treatment for NAFLD progression.

Method: Male Sprague Dawley rats were fed either a high-fat high-cholesterol (HFHC) diet to induce NASH
or normal chow for 16 weeks. Rats in the HFHC group were randomized to receive the Adra2aR antagonist Yohimbine hydrochloride (YoHCl) (titrated to 0.4mg/kg daily) in drinking water for the final 8 weeks. Subsequently, formalin fixed liver sections were stained with HandE and picrosirius red. The collagen proportionate area (CPA) was measured and fibrogenic and pro-inflammatory changes were investigated in liver tissue using qPCR, ELISA, and CD68 immunohistochemistry.

Results: HFHC diet increased liver/body weight ratio (6.5 ($\pm$ 0.9) vs. 2.8 ($\pm$ 0.3); $p < 0.0001$) and NAS score (5.8 ($\pm$ 0.4) vs. 0.1 ($\pm$ 0.3); $p < 0.0001$), which were both reduced by YoHCl (5.7 ($\pm$ 0.4); $p = 0.03$ and 4.3 ($\pm$ 1.1); $p = 0.001$, respectively). Furthermore, the HFHC fed animals had higher CPA (15.0 (13.0-19.5) vs. 1.4 (0.6-1.5)%; $p <0.01$), and YoHCl treatment significantly reduced this (7.6 (4.1-9.2)%; $p <0.01$). Moreover, the gene expression of TIMP1 was increased in HFHC animals and significantly reduced by YoHCl (8.4 (6.1-9.2) vs. 13.5 (9.2-25.8), $p = 0.03$). The HFHC diet increased CD68 stained areas ($p < 0.0001$) as well as gene expression of chemokines (Ccl3, Cx3Cl1, CxCl1, CxCl5; all $p < 0.001$) and protein levels of CxCl5 and Cx3Cl1. Importantly, CD68 stained area ($p = 0.03$), chemokine gene expressions, and liver tissue levels of Cxcl5 and Cx3cl1 were all reduced by YoHCl indicating a reduction in hepatic inflammation.

Conclusion: This study demonstrates that Adra2aR antagonism reduces fibrosis and inflammatory progression in NAFLD, associated with reduced immune activation. This suggests Adra2aR antagonism may slow progression of NAFLD. The potential for therapeutic translation in NAFLD patients warrants further investigation.

P04-12YI Early diagnosis of fibrosis in non-alcoholic fatty liver disease

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Background and aims: Study the relationship between impaired lipid metabolism and the degree of fibrosis in patients with non-alcoholic fatty liver disease (NAFLD).

Method: The study included 58 patients aged from 32 to 60 years, who were divided into two groups by randomization. Group 1 included 32 patients with isolated course of NAFLD; in patients of group 2 (26 patients), NAFLD was associated with type 2 diabetes mellitus (DM). In all patients, the diagnosis was verified using instrumental (ultrasound) and clinical and laboratory research methods. To exclude viral etiology of liver damage, hepatitis B and C markers were determined by PCR. The functional state of the liver was assessed by the level of total bilirubin and its fractions, alkaline phosphatase (alkaline phosphatase) activity, AST and ALT, thymol sample, lipid profile. Also determined the level of platelets in the serum. The risk of developing fibrosis was calculated using the NFS formula.

Results: When assessing the trophological status in both groups, patients with overweight prevailed. The body mass index was less than 25 kg/m 2 in only 17.5% of patients in the group with an isolated course of NAFLD and only in 5% of patients in the group with a combination of NAFLD and type 2 diabetes, while grade 2 obesity was observed in 7% and 50 % of patients in each group, respectively. Disruption of lipid metabolism was detected in patients of the two groups. Total cholesterol, β-lipoproteins and triglycerides were significantly increased in both groups ($p < 0.05$), however, in the 2nd group, the levels of these indicators are higher than in the NAFLD group ($p < 0.05$). HDL was significantly reduced ($p < 0.001$) in the observation groups, due to high serum triglyceride levels. However, a decrease in the level of HDL in the 2nd group was found in 30% of patients compared with an isolated course of NAFLD (22%). Significant differences in levels of LDL and VLDL in the 1st and 2nd groups could not be identified. Thymol samples, reflecting the change in the colloidal composition of proteins in serum, were significantly ($p < 0.05$) increased in the 1st group of patients and in the 2nd group when compared with the 1st group ($p < 0.05$). Correlation the analysis revealed positive relationships between the BMI and the TG level ($r = 0.64$; $p < 0.001$) in the NAFLD group. The increase in the concentration of TC and TG in the group of patients with NAFLD and type 2 DM was directly dependent on BMI ($r = 0.67$, $p < 0.05$; $r = 0.71$, $p < 0.05$, respectively).

Conclusion: NAFLD is a chronic progressive disease that leads to the development of liver cirrhosis and hepatocellular carcinoma. The NAFLD fibrosis score test is a diagnostic non-invasive test to determine liver fibrosis and disease progression. In NAFLD, lipid metabolic disturbances correlate with the severity of liver fibrosis: the higher the dyslipidemia, the greater the likelihood of developing fibrosis.

P04-13 S-adenosylmethionine alleviates hepatic steatosis via alternation of lipid- and bile acid-metabolism and microbiota

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Email:

Background and aims: S-adenosylmethionine (SAM) is important as a methyl donor during methylation and is involved in production of N^1-methylnicotinamide (MNAM), polyamine, phosphatidylcholine, and sarcosine designated as the "one-carbon cycle". These methylated products may have an important role in the development of NAFLD, and we previously reported that MNAM as well as nicotinamide (NAM) improved high-fat diet (HD)-induced NAFLD in mice (EASL ILS 2019 and NASH summit 2018). Furthermore, SAM administration has been also reported to improve NAFLD (Dahlhoff C, et al. Mol Metab. 2014;3:565). Therefore, we were prompted to investigate the mechanisms of how SAM improves NAFLD more in detail.

Method: C57BL/6J mice (n = 20) were divided into 4 groups and fed with normal diet (ND); high-fat diet containing fat of 40% (HFD); ND+SAM (SAM mixed with ND to 0.1% wt/wt); and HFD+SAM for 8 weeks. Liver SAM-related metabolites (Fig. 1) were checked by LC/MS, and the expression of lipid and bile acid metabolism-related genes in the liver and the ileum was analyzed by RT-PCR. Microbiota analysis was performed by T-RFLP analysis. Metabolized products by microbiota, such as short-chain fatty acids in serum were also measured by LC/MS.

Results: SAM suppressed body weight gain induced by HFD with histological improvement of steatosis without affecting food intake. Mice treated with SAM increased nicotinamide mononucleotide (NMN) content in the liver, leading the activation of Sirtuins. In contrast, SAM reduced glycine, sarcosine, and spermidine content. Interestingly, SAM completely suppressed the HFD-upregulated expression of genes involved in cholesterol (HMG-CoAR), fatty acid (FAS), and bile acid synthesis (CYP7A) in the liver. Furthermore, SAM also decrease the HFD-increased expression of ASBT and FGF15 in the ileum. Finally, SAM significantly reduced Firmacutes, but oppositely increased Actinobacteria at phylum level, accompanying the increase in serum lactic acid level.

Conclusion: The improvement of fatty liver by SAM was probably mediated via several pathways including NAD-Sirtuins, sarcosine, and spermidine, leading to the alternation in lipid- and bile acid-metabolism and microbiota. Finally, SAM did not show toxicity, and is sold as health supplementation; therefore, we believe it has promising potentials in treating NAFLD.

Figure:

Methionine → S-adenosylmethionine (SAM)

Spermidine

Nicotinamide (NAM) → NMN → NAD

Sirts

Nicotinamide N-methyltransferase (Nnmt)

Glycine

Ethanolamine

Phosphatidylethanolamine

Sarclsin (methylglycine)

N^1-methylnicotinamide (MNAM)

Taurine

Homocysteine

Betaine (Trimethylglycine)

Dimetylglycine

Methionine

Phosphatidylcholine → S-adenosylhomocysteine

S-adenosylmethionine (SAM)

P04-14YI Moderate alcohol consumption is associated with higher grade of liver fibrosis in patients with non-alcoholic fatty liver disease

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Background and aims: A moderate daily alcohol consumption (under 30 g for men and 20 g for women) has been considered safe in patients with non-alcoholic fatty liver disease (NAFLD) but no evidence supports its strongly.
The aim of our study was to evaluate if a moderate alcohol consumption, lower than that defining an alcoholic etiology of liver disease, may influence liver disease in patients with NAFLD.
Method: We enrolled 175 consecutive patients with clinical diagnosis of NAFLD from January 2015 to May 2019. Each patient underwent liver stiffness measurement with 2D liver shear wave elastography (Aixplorer, SuperSonic Imagine) and a detailed anamnesis with a questionnaire on current and lifetime alcohol consumption.
The questionnaire allowed us to generate a new indicator for lifetime alcoholic cumulative units (LACU): median alcohol units in a week/7 per drinking year.
We decided to use the weekly units divided by 7 (days in a week) and not the daily units, as in our cohort alcohol consumption frequently varied during the week, with a higher use during the weekend; we also decided to include the time variable (paralleling pack/years for cigarette smoking) to obtain an evaluation of cumulative lifetime risk.
We thus divided the population into three groups according to liver fibrosis: absence of significant fibrosis (group A, <7.1 KPa, n = 120), moderate or severe fibrosis (group B, 7.1-12.9 KPa, n = 34), cirrhosis (group C, ≥13 KPa, n = 19).
Results: Median current alcoholic consumption (weekly units) was 1 (interquartile range IQR 0-3) in group A, 3 (IQR 0-10) in group B and 0 (IQR 0-2) in group C with a significant difference between A and B (p = 0.032). Group C showed no significant differences with both groups.
Median LACU was 3.6 (IQR 0-8.6) in group A, 17.9 (IQR 2.5-42.9) in group B and 2.9 (IQR 0-30) in group C with an even more significant difference between A and B (p = 0.002). Group C showed no significant differences with both groups.
Result are resumed in figure 1.
Conclusion: A commonly considered safe alcohol consumption is associated with significant fibrosis in patients with NAFLD, as a possible expression of a synergic hepatotoxic effect of alcohol on an already distressed liver. Though preliminary, from our data arises the hypothesis that it is not possible to safely define a risk-free dose of alcohol consumption in NAFLD patients.

Figure:
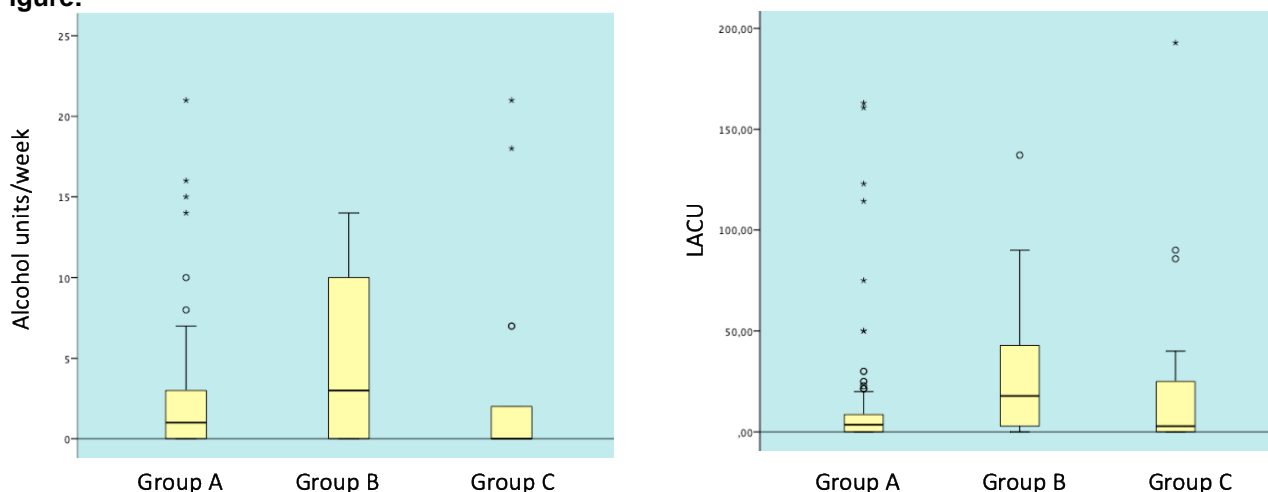
P04-15 Circulating pcsk9 levels correlated with advanced disease in patients with biopsy-proven non-alcoholic fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) has been associated with cardiovascular events, mainly patients with NASH and fibrosis. Proprotein convertase subtilisin/kenin type 9 (PCSK9) is secreted into the plasma by the liver and regulates lipid homeostasis by promoting degradation of the LDL receptor and possibly lipogenesis, disrupting cholesterol homeostasis. The aim of the study was to evaluate levels of circulating PCSK9 in patients with NAFLD.

Method: Sixty-four NAFLD biopsy-proven patients were included. Liver biopsies were classified by SAF score as Non-alcoholic fatty liver (NAFL) (n = 24) or as non-alcoholic steatohepatitis (NASH) (n = 40). Levels of circulating PCSK9 were evaluated by ELISA in 50 μl of serum samples.

Results: Mean age was 48 ± 14 in the NAFLD group and 54 ± 10 in the NASH group (p = 0.04). Male were el 36% in the NAFL group and 52% in the NASH (p = 0.268).

Levels of triglycerides were significantly higher in NASH compared to NAFL (180.4 ± 81.2 vs. 114.7 ± 56.2; p = 0.001) and levels of HDL cholesterol were higher in NAFL in comparison with NASH patients (60.3 ± 12.3 vs. 41.2 ± 10.3; p < 0.001) while levels of LDL cholesterol and total cholesterol were similar between both groups (0.385 and 0.225 respectively).

PCSK9 in NASH patients was significantly higher than in NAFL group (453.11 ± 164.5 vs. 301 ± 127.7; p < 0.001). Patients without inflammation (n = 6; 9.8%) had lower levels of PCSK9 than those with mild (n = 29, 47.5%) or moderate grade of inflammation (n = 16; 26.2%) (292 ± 96 vs. 327.9 ± 157.9 vs. 462.4 ± 168); p = 0.014). On the other side, PCSK9 was lower in patients without ballooning (n = 21, 42%) than in those with mild (n = 24; 48%) or significant ballooning (n = 5; 20.8% (p = 0.001). Indeed, fibrosis strongly correlated with PCSK9 levels (p = 0.028; r = 0.560), patients with fibrosis had higher levels of PCSK9 than those without (428.3 ± 162 vs. 324.3 ± 172; p = 0.04)

Conclusion: Circulating PCSK9 levels correlated positively with fibrosis stage, ballooning and inflammation degree in patients with biopsy-proven NAFLD. PCSK9 could be a link between advanced NAFLD and cardiovascular risk.

Figure:
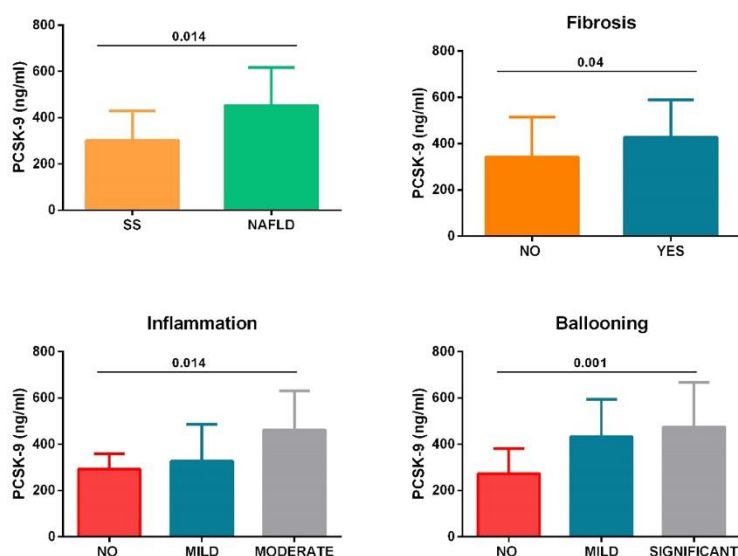
P04-16YI The co-stimulatory signals mediated by icos-icosl dyad promote the evolution of non-alcoholic steatohepatitis (NASH)

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Background and aims: Growing evidence indicates a role of adaptive immunity in sustaining hepatic inflammation during the evolution of NASH. However, the signals regulating B- and T-lymphocyte responses are still poorly characterized. The Inducible T-cell Co-Stimulator (ICOS) and its ligand ICOSL (B7h) are members of the B7/CD28 family and play multiple roles in immunity by regulating T-cell activation/survival and antibody production. In this study, we have investigated the possible involvement of ICOS-ICOSL dyad in NASH evolution.

Method: ICOS and ICOSL were investigated in experimental model of NASH based on mice feeding with choline-deficient amino acid sufficient (CDAA) or choline/methionine deficient (MCD) diets as well as in the sera of 40 NASH patients.

Results: Soluble ICOS and ICOSL were significantly increased in the sera of NASH patients and soluble ICOSL positively correlated with the titres of IgG against oxidative stress derived antigens (OSE). In different animal models of NASH, the liver expression of ICOS was down-modulated while that ICOSL increased in a time-dependent manner in parallel with the development of anti-OSE B- and T-cell activation and NASH progression to fibrosis. Mice deficient for ICOSL receiving the MCD diet for 6 weeks had milder steatohepatitis than wild type animals and reduced B- and T-cell responses. Furthermore, the lack of ICOSL prevented the development of fibrosis by affecting osteopontin production by hepatic macrophages.

Conclusion: Altogether these data indicate that not only ICOS-ICOSL dyad play a role in modulating adaptive immunity during NASH evolution but also directly influences pro-fibrogenic mechanisms suggesting these costimulatory molecules as a possible target for therapeutic interventions.

P04-17 FAST score for identification of patients with non-alcoholic steatohepatitis (NASH), NAS≥4 and significant (F≥2) or advanced (F≥3) fibrosis

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Background and aims: Given the development of drugs for at-risk NASH patients, the FAST™ score combining liver stiffness measurement (LSM) and controlled attenuation parameter (CAP) by FibroScan and AST was established to identify patients with active NASH (NAS≥4) and significant fibrosis (F≥2). This study compares the performance of FAST in its derivation and seven external validation cohorts using liver biopsy as the reference standard.

Method: *Derivation cohort:* Prospective study of patients with suspected NASH from 7 tertiary British centres. *External validation cohorts:* (A) Patients screened for NAFLD during colonoscopy in one US center, (B-F) Patients from tertiary liver centers in France, Malaysia, China, Hong Kong and Turkey, respectively, (G) Patients with severe obesity (BMI>35 kg/m²) assessed at the time of bariatric surgery in a single French centre. LB were read in a blinded manner either by a single local pathologist (cohorts B-F) or in a double blinded manner with consensus by two expert pathologists (derivation, A, G). FAST performances to identify NASH+NAS≥4+F≥2 (initial target) and NASH+NAS≥4+F≥3 were assessed using area under the receiver operating characteristics (AUROC) and associated 95% confidence interval.

Results: Characteristics and performances in each cohort and pooled validation cohort are shown in the Table.

Conclusion: FAST showed good to excellent performance in the derivation and validation cohorts. FAST is a promising tool for identifying patients with NASH+NAS≥4+F≥2 and NASH+NAS≥4+F≥3 that may be eligible for clinical trials and/or pharmacotherapy.

Figure:

Cohorts	Derivation	External Validation							
		A - USA	B - France	C - Malaysia	D – China WZ	E – China HK	F - Turkey	G - Bariatric	Pooled
N	350	242	182	176	104	83	129	110	1026
Age (y)	54 (45-63)	55 (50-60)	58 (49-66)	52 (46-60)	41 (30-50)	55 (46-63)	49 (38-57)	41 (33-50)	52 (44-60)
Female	149 (43%)	97 (40%)	65 (36%)	84 (48%)	28 (27%)	42 (51%)	59 (46%)	88 (80%)	463 (45%)
BMI (kg/m^2)	34 (30-39)	34 (30-36)	32 (29-37)	28 (26-30)	26 (23-28)	29 (26-32)	33 (30-36)	43 (39-47)	31 (28-36)
AST (IU/L)	36 (27-52)	22 (18-27)	36 (28-50)	38 (29-62)	34 (27-52)	41 (28-59)	37 (28-59)	26 (21-39)	32 (23-48)
LSM (kPa)	8.9 (6.2-13.9)	6.0 (4.7-8.2)	7.9 (5.9-11.5)	7.0 (6.0-10.0)	5.8 (5.1-6.7)	8.8 (6.6-12.2)	11.1 (8.6-14.6)	5.9 (4.7-8.8)	7.2 (5.3-10.3)
CAP (dB/m)	342 (307-373)	317 (276-360)	326 (297-369)	323 (289-343)	316 (284-332)	319 (290-354)	329 (304-356)	310 (275-374)	321 (288-355)
NASH+NAS≥2+F≥2	174 (50%)	28 (12%)	78 (43%)	36 (20%)	9 (9%)	36 (43%)	74 (57%)	16 (15%)	277 (27%)
AUROC (95% CI)	0.80 (0.76-0.85)	0.86 (0.80-0.93)	0.80 (0.73-0.86)	0.85 (0.78-0.91)	0.84 (0.73-0.95)	0.85 (0.76-0.93)	0.74 (0.65-0.82)	0.95 (0.91-0.99)	0.85 (0.83-0.87)
NASH+NAS≥2+F≥3	108 (31%)	10 (4%)	49 (27%)	26 (15%)	3 (3%)	26 (31%)	45 (35%)	10 (9%)	169 (16%)
AUROC (95% CI)	0.79 (0.74-0.84)	0.88 (0.79-0.98)	0.83 (0.77-0.89)	0.83 (0.75-0.92)	0.88 (0.78-0.99)	0.82 (0.71-0.92)	0.73 (0.64-0.83)	0.94 (0.89-0.99)	0.86 (0.83-0.89)

Distribution: Median (Q1-Q2) or figure (%).

P04-18 Ablation of High mobility group box-1 in intestinal epithelial cells causes intestinal lipid accumulation and reduced non-alcoholic steatohepatitis

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Background and aims: non-alcoholic steatohepatitis (NASH) is a metabolic disorder where impaired gut-liver interaction can contribute to liver steatosis. Previous work form our laboratory has established that High mobility group box-1 (HMGB1), a damage-associated molecular pattern, participates in liver disease. HMGB1 levels increase in serum from patients with NASH; however, its role in NASH has not been investigated. Since HMGB1 is expressed in intestinal epithelial cells (IEC), we hypothesized that IEC-derived HMGB1 could play a role in NASH by regulating hepatic steatosis.

Method: mice with conditional ablation of *Hmgb1* in IEC (*Hmgb1*$^{\Delta IEC}$) and control littermates were fed a high fat, cholesterol and fructose enriched diet (HFCFD) or equicaloric control diet (CD) for 1 week or 6 months and parameters of hepatic and intestinal injury were analyzed

Results: *Hmgb1*$^{\Delta IEC}$ mice are protected from HFCFD-induced NASH after 1 wk or 24 wk of feeding and display lipid droplet accumulation in IEC, increased triglyceride (TG) and cholesterol (CHO) concentrations in IEC, and decreased TG and other lipid species in serum. Olive oil (OO) and cholesterol gavage resulted in decreased serum TG and CHO in *Hmgb1*$^{\Delta IEC}$ mice, respectively, indicating delayed and reduced chylomicron release. ApoB48 and *Mttp* were downregulated in *Hmgb1*$^{\Delta IEC}$ mice fed HFCFD suggesting decreased lipid packaging and chylomicron formation.

Conclusion: ablation of *Hmgb1* in IEC results in downregulation of ApoB48 and *Mttp* leading to lipid accumulation in IEC, decreased chylomicron release, reduced serum TG and less lipid accumulation in hepatocytes from *Hmgb1*$^{\Delta IEC}$ mice.

Figure:
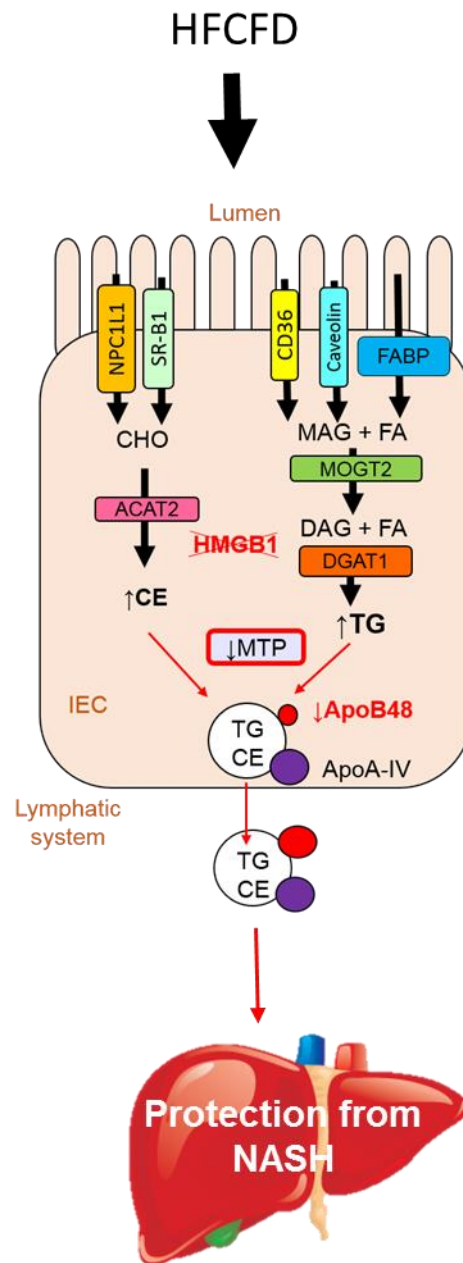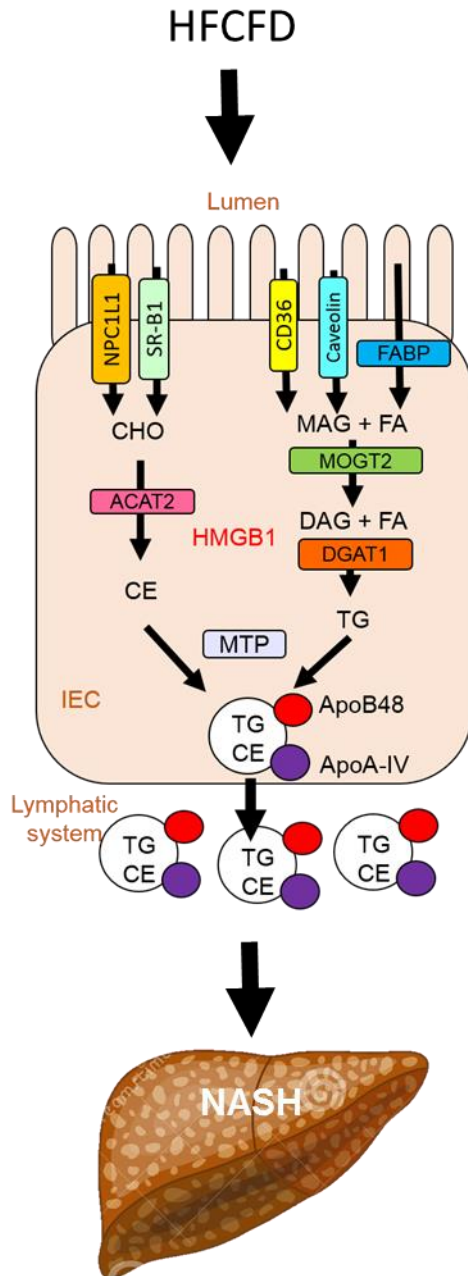
P04-19YI Fibrosis impact on quality of life in non-alcoholic fatty liver disease (NAFLD)

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Background and aims: The presence of advanced histological lesions in NAFLD could be associated with worse quality of life. We therefore analyzed whether there were differences in quality of life in patients with biopsy-proven NAFLD based on the presence or absence of NASH and significant fibrosis.

Method: A group of 256 patients, made up of 138 men and 118 women, with a mean age of 56.42 (SD = 11.51 years) was selected. This group was divided into four subgroups by severity, according to steatosis, activity, and fibrosis (SAF) score: G_1 (NAFLD without significant fibrosis; n = 70), G_2 (NASH with significant fibrosis; n = 66), G_3 (NAFLD with significant fibrosis; n = 87), and G_4 (NASH without significant fibrosis; n = 33). All of them were evaluated using the following instruments: psychosocial interview, 12-Item Short-Form Health Survey (SF-12v.2), and Chronic Liver Disease Questionnaire-Non-Alcoholic Fatty Liver Disease (CLDQ-NAFLD). Snedecor's F and Welch's U were computed as omnibus tests to compare the quality of life between groups, and the Tukey HSD and Games-Howell were applied for post hoc multiple comparisons. Cohen's d was used as an index of effect size.

Results: There were differences in the total scores between groups in the physical component summary measured with the SF-12v.2 ($p = 0.000$), and total CLDQ-NAFLD ($p = 0.000$). In the specific dimensions, the most relevant differences (medium and large effect size) were found between the following groups: 1) G_1-G_2, with higher scores in G_1 in physical functioning ($p = 0.010$, $d = 0.563$), role-physical ($p = 0.001$, $d = 0.652$), bodily pain ($p = 0.008$, $d = 0.558$), general health ($p = 0.003$, $d = 0.616$), vitality ($p = 0.003$, $d = 0.603$), activity ($p = 0.003$, $d = 0.616$), fatigue ($p = 0.000$, $d = 0.754$), and systemic symptoms ($p = 0.001$, $d = 0.676$), 2) G_1-G_3, with higher scores in G_1 in physical functioning ($p = 0.002$, $d = 0.580$), activity ($p = 0.001$, $d = 0.636$), fatigue ($p = 0.004$, $d = 0.540$), systemic symptoms ($p = 0.000$, $d = 0.683$), and worry ($p = 0.001$, $d = 0.609$), 3) G_2-G_4, with lower scores in G_2 in role-physical ($p = 0.017$, $d = -0.626$), bodily pain ($p = 0.004$, $d = -0.723$), activity ($p = 0.020$, $d = -0.601$), emotional, ($p = 0.035$, $d = -0.618$), fatigue ($p = 0.001$, $d = -0.930$), and systemic symptoms ($p = 0.001$, $d = -0.784$), and 4) G_3-G_4, with lower scores in G_3 in bodily pain ($p = 0.033$, $d = -0.534$), activity ($p = 0.008$, $d = -0.621$), fatigue ($p = 0.008$, $d = -0.685$), systemic symptoms ($p = 0.001$, $d = -0.796$), and worry ($p = 0.012$, $d = -0.570$).

Conclusion: Patients with significant fibrosis had a lower quality of life than those without significant fibrosis, regardless of presence or absence of NASH.

Figure:

P04-20 Metabolic caracterization of hepatocellular cancer cells related to non-alcoholic fatty liver disease

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Background and aims: The raise in incidence of hepatocellular carcinoma (HCC) has been associated in non-alcoholic fat liver disease (NAFLD). The metabolic-related-markers play a role in the maintenance of the hyper-glycolytic acid- resistant phenotype of cancer, contributing to the evolution and to its aggressiveness. We sought to investigate the expression of several metabolic markers in NAFLD-related HCC patients associated to and related these factors to their clinical- pathological characteristics.
Method: We evaluated 35 HCC specimens from 21 patients diagnosed with NAFLD undergoing liver resection (n = 13) or liver transplantation (n = 9). One patient was submitted to resection and afterwards to transplant. We compared histological features, clinical aspects, demographic and biochemical data and immunohistochemical reactivity for monocarboxylate transporters (MCT) 1, 2 and 4, the chaperone CD147, carbonic anhydrase IX and glucose transporter-1 (GLUT1).
Results: Cirrhosis was present in 12 of the 21 patients, (7 F4A x 4F4B x 1F4C, Laennec Staging), 8 of them presenting Child A and 4 Child B cirrhosis. Among the 9 patients without cirrhosis, 3 patients presented NASH F3 and 6 patients had NASH F2. Ages ranged from 50 to 77 years and 16 patients were male (76%). Sixteen patients (76%) had diabetes mellitus, 17 patients (81%) had arterial hypertension and 19 patients (90%) had BMI above 25kg/m^2. Only 8 patients (38%) had dyslipidemia. Alpha-fetoprotein level was normal in 13 patients. Thirteen nodules were well-differentiated HCC, G1/G2, whereas 22 were poorly differentiated (G3/G4). Steatosis and ballooning was present in 26 and in 31 nodules, each of them remarkable in 9 and in 22, respectively. According to the current criteria 25 were subtyped as "steato-hepatitic". The expression of MCT4 was higher in nodules with extensive intratumoral fibrosis (3 or 4/4) and its expression in the plasma membrane was higher in nodules with more advanced clinical stages (BCLC B or C). GLUT1 expression was marked in nodules with extensive intratumoral steatosis (G2/G3), in those with higher intratumoral fibrosis (G3/G4) and in nodules that occurred in liver with NASH parenchymal activity G.2/G.3 and with more marked hepatocellular ballooning (G2). GLUT1 expression was also higher in nodules from patients with more advanced clinical stages (BCLC B or C).
Conclusion: i. NASH-related HCC can arise in patients without cirrhosis. **ii.** Histological markers of "steato-hepatitic HCC" and high architectural and nuclear degrees were the morphological alterations more frequently found; **iii.** The expression of MCT4 and GLUT1, markers of glycolytic metabolic phenotype, were higher in HCC with BCLC B or C. The expression of GLUT1 correlated with higher degrees of steatosis, marked ballooning, intratumoral fibrosis and higher parenchymal activity, all of them potential features associated with poor prognosis.

P04-21YI Genotyping rs738491 in the SAMM50 Gene May Increase Accuracy of Non-invasive Assessment for Non-alcoholic Steatohepatitis

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Background and aims: Inconsistencies in non-invasive assessments of non-alcoholic steatohepatitis (NASH) could be attributed to lack of consideration for genotyping. We aim to explore the difference in diagnostic efficacy of biomarkers under respective genotypes of rs738491.

Method: 349 (163 NASH and 186 non-NASH) biopsy-proven NAFLD subjects were included. NASH was defined as NAS ≥5. Genotyping of rs738491 was obtained by MassARRAY reactions. Area under operating characteristics (AUROC) for transaminase (ALT), cytokeratin-18 (CK-18) M30, and CK-18 M65 were calculated for NASH under genotypes of rs738491.

Results: Difference in diagnostic ability of biomarkers for NASH were found in that CK-18 M30 and M65 exhibited ideal AUROC for the wildtype (CC) under rs738491 (0.850, 0.860; respectively), while for its mutants (CT+TT) subpar AUROC was obtained (0.682, 0.670; respectively). Discrepancies were also found among optimal cut-offs for discriminating NASH. Optimal cut-offs for ALT were 55 U/L and 69 U/L for wildtype and mutants, respectively, while for CK-18 M65 were 287 U/L and 228 U/L, respectively.

Conclusion: This suggest hepatologists should consider genotype prior to non-invasive testing in order to limit misdiagnosis of NASH. Discrepancies exist for non-invasive methods to diagnose NASH, but genotyping genetic variants may serve to decrease such error and to increase diagnostic accuracy.

Figure:
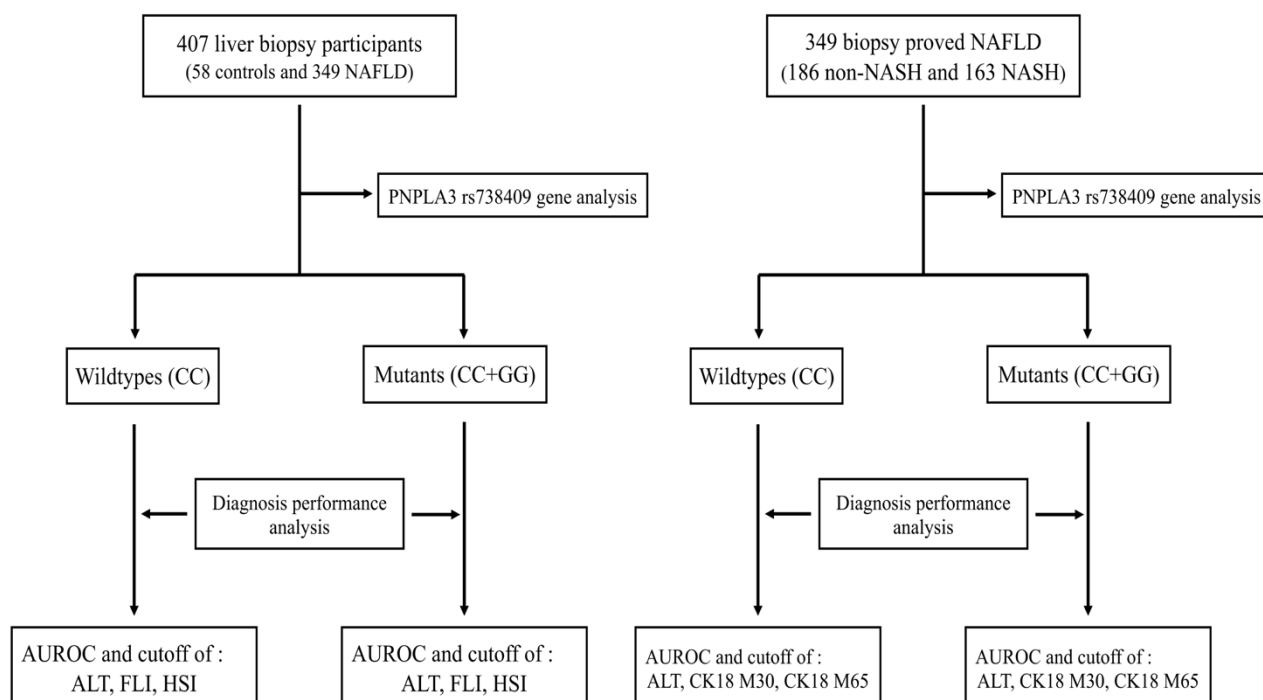
Table 3. Diagnostic performance of noninvasive biomarker and panels of NAFLD						
Noninvasive biomarker and panels	AUC	95% CI	P-value	Cutoff	Sensitivity	Specificity
ALT						
All Patietnts	0.768	0.724 to 0.808	<0.0001	37 U/L	72.49 (67.5 - 77.1)	70.69 (57.3 - 81.9)
CC	0.646	0.553 to 0.732	0.0285	27 U/L	78.72 (69.1 - 86.5)	50 (29.1 - 70.9)
CG+CG	0.844	0.795 to 0.885	<0.0001	43 U/L	68.35 (62.0 - 74.2)	87.5 (71.0 - 96.5)
Fatty liver index						
All Patietnts	0.724	0.677 to 0.768	<0.0001	50	69.88 (64.6 - 74.8)	71.43 (57.8 - 82.7)
CC	0.59	0.495 to 0.681	0.1873	42	77.17 (67.2 - 85.3)	52.17 (30.6 - 73.2)
CG+CG	0.819	0.766 to 0.864	<0.0001	50	70.22 (63.8 - 76.1)	80.65 (62.5 - 92.5)
Hepatic steatosis index						
All Patietnts	0.761	0.707 to 0.808	<0.0001	37	71.37 (65.3 - 76.9)	72.09 (56.3 - 84.7)
CC	0.667	0.572 to 0.754	0.0112	37	72.04 (61.8 - 80.9)	66.67 (41.0 - 86.7)
CG+CG	0.841	0.779 to 0.892	<0.0001	37	75.97 (68.4 - 82.5)	79.17 (57.8 - 92.9)

Table 4. Diagnostic performance of noninvasive biomarker and panels of NASH						
Noninvasive biomarker	AUC	95% CI	P-value	Cutoff	Sensitivity	Specificity
ALT						
All Patietnts	0.755	0.706 to 0.799	<0.0001	57 U/L	71.17 (63.6 - 78.0)	70.97(63.9 - 77.4)
CC	0.775	0.677 to 0.855	<0.0001	55 U/L	70.27 (53.0 - 84.1)	75.44(62.2 - 85.9)
CG+CG	0.745	0.685 to 0.799	<0.0001	61 U/L	69.83 (60.6 - 78.0)	71.9(63.0 - 79.7)
CK18 M30						
All Patietnts	0.724	0.672 to 0.772	<0.0001	183 U/L	68.21 (60.1 - 75.5)	70.18(62.7 - 76.9)
CC	0.814	0.717 to 0.890	<0.0001	172 U/L	80 (63.1 - 91.6)	75(61.1 - 86.0)
CG+CG	0.688	0.622 to 0.748	<0.0001	196 U/L	62.96 (53.1 - 72.1)	70.18(60.9 - 78.4)
CK18 M65						
All Patietnts	0.719	0.666 to 0.767	<0.0001	228 U/L	74.83 (67.1 - 81.5)	67.25(59.7 - 74.2)
CC	0.813	0.715 to 0.888	<0.0001	217 U/L	80 (63.1 - 91.6)	71.15(56.9 - 82.9)
CG+CG	0.678	0.612 to 0.739	<0.0001	207 U/L	75 (65.7 - 82.8)	62.28(52.7 - 71.2)

P04-22 Results of life style modification on weight loss and factors of failure in NAFLD

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Background and aims: Treatment of non-alcoholic fatty liver disease (NAFLD) is essentially based on life style modification: diet and physical activity and managing risk factors. Weight loss of at least 3-5% has proven to improve steatosis and 7-10% to improve fibrosis.
Objective: To assess weight loss after 3 and 6 months of life style modification

Method: Prospective study conducted over one year (December 2017 to December 2018) including all patients followed for NAFLD. All patients have been educated about diet and physical activity, and have been informed about complications and risks of the metabolic syndrome.

Results: We included 58 patients: 46 women and 12 men (sex-ratio = 0.2) with an average age of 49.4 years [14-81 years]. Type 2 diabetes was found in 22 patients (37.9%), high blood pressure in 20 patients (34.5%) and dyslipidemia in 24 patients (41.4%). The average body mass index was 32.6 Kg/m2 [20-55 kg/m2]. Seventeen patients (29.1%) were overweight and 35 (60.6%) patients were obese. The average waist circumference was 104.24 cm [67-138cm]. Android obesity was objective in 46 patients (79.3%) and hepatic cytolysis was found in 9 patients (15.5%).
The average weight loss at 3 months was 1.26% [0.8-10%] and at 6 months was 2.33% [0.9-13.6%].
After 6 months of follow-up: 38 patients (65.5%) lost weight, 13 patients (22.4%) gained weight and 7 patients (12.1%) maintained a stable weight. Only 8 patients had a weight loss ≥5%.
Normalization of hepatic cytolysis was noted in 66.6% of patients who lost weight and had initial cytolysis.
Ischemic heart disease, gonalgia and advanced age were factors of non-adherence in patients who had not lost weight.

Conclusion: Life style modification allowed weight loss in 65.5% of patients and normalization of hepatic cytolysis in 65.5% of cases. Non-adherence to the treatment was related to the impossibility to perform regular physical activity.

P04-23YI Suboptimal metabolic control is a risk factor for liver disease progression in patients with type 2 diabetes mellitus

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Background and aims: Type-2 diabetes mellitus (T2DM) is associated with liver disease progression (LDP). The risks of LDP, including metabolic control and obesity, have not been adequately studied in T2DM patients.

Method: We conducted a retrospective cohort study among all adult patients diagnosed with T2DM at Cochin University Hospital (Paris, France) from 2010 to 2018 and with at least two HbA1c measurements (N = 6, 806). Outcome measure was progression of the FIB-4 index to the threshold of 3.25, a surrogate of advanced fibrosis in T2DM patients (1). We excluded patients with an extrahepatic cause of thrombocytopenia, including extra-hepatic cancer, immunosuppression, and connective tissue disorders. Glucose metabolism was estimated with HbA1c variations and categorized as controlled (HbA1c fluctuations <2.5%) or uncontrolled (HbA1c fluctuations ≥2.5%). We used Cox models with age as time-scale to compute lifetime hazard ratios for LDP associated with sex, liver-related risk factors, metabolic control and obesity.

Results: The average (95% CI) age at cohort inception was 60.9 (60.6-61.2) years and 58.5% were males. The prevalence (95% CI) of hepatic risk factors and obesity were 22.8% (21.8%-23.8%) and 48.7% (47.5%-49.9%), respectively. The mean (95% CI) FIB-4 index and HbA1c levels were 1.92 (1.80-2.05) and 7.82 (7.78-7.87). A total of 974 (13.5%) patients progressed to a FIB-4≥3.25 over the 11, 831 person-year observational period. Mean age at LDP was 78.8 (77.8-92.2) and 81.0 (83.2-84.8) years in patients without and with metabolic control, respectively (p = 0.002 with log-rank test, see figure). The hazard ratios (95% CI) for LDP associated with female sex, presence of a liver-related risk factors and suboptimal metabolic control were 0.68 (0.59-0.78), 2.55 (2.22-2.90) and 1.47 (1.12-1.91), respectively. Obesity was not associated with LDP.

Conclusion: In a hospital sample for T2DM patients, suboptimal metabolic control was associated with liver disease progression.

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Figure:

Risk of liver disease progression by metabolic control in a retrospective cohort of T2DM patients (n=6802)
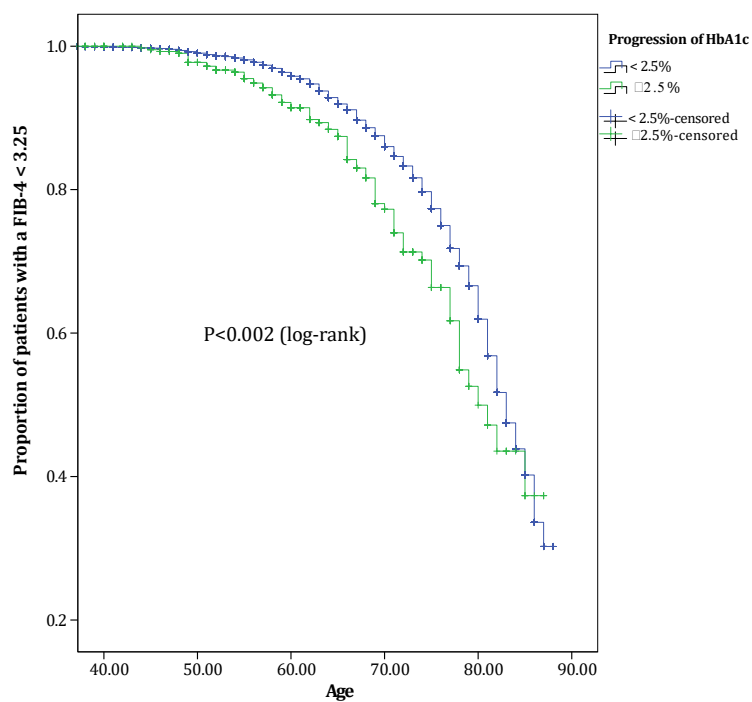
P05-01YI Alcohol and other contributing factors to the burden of liver disease among patients with type-2 diabetes mellitus: a retrospective longitudinal study

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Background and aims: Obesity and type-2 diabetes mellitus (T2DM) are thought to account for a substantial number of liver-related complications in high-income countries. The relative contribution of traditional risk factors of liver-disease progression is not reported in T2DM patients.

Method: We conducted a retrospective cohort study among all adult patients discharged from Cochin University Hospital (Paris, France) between 2010 and 2018 with an ICD-10 code for T2DM (N = 26, 569) and recorded all liver-related complications, including decompensated cirrhosis and primary liver cancer, and all liver-related and non-liver-related risk factors. (1) We measured with population attributable fractions the contribution to liver disease progression of the following risk factors: alcohol use disorders (AUDs), chronic viral hepatitis, other causes of chronic liver diseases (autoimmune disease, iron and copper storage disorders, alpha antitrypsin deficiency), extra-hepatic cancer, immunodepression and obesity.

Results: Mean (95% CI) age at cohort inception was 64.4 (64.2-64.5) years overall, and 57.7% were males. The prevalence (95% CI) of traditional risk factors of liver disease progression, including alcohol use disorders (AUDs: 11.4%), chronic hepatitis C (1.5%), chronic hepatitis B (0.7%), chronic hepatitis D (0.0%) other causes of chronic liver disease (1.3%), was 14.2% (13.8%-14.6%). The prevalence of extra-hepatic cancer, HIV/Aids; and other risk factors (connective tissue disorders and transplant recipient) were 10.9%, 0.4% and 0.7%, respectively. Overall, 397 (1.5%) liver disease progressions were recorded over the 20, 669 person-year observational period, corresponding to a person-time incidence rate of 19 cases per 1000 person-year. The attributable fraction of all traditional risk factor of liver disease progression was 74%; AUDs contributed to more than 50% of the burden (figure). The contribution of obesity and of immunodepression was not statistically significant. Extra-hepatic cancer contributed slightly to the burden.

Conclusion: In a large population of T2DM patients, traditional risk factors of liver disease progression, and not obesity, contributed to three-fourth of the burden of liver disease progression. Alcohol use disorders contributed, by themselves, to more than half of the burden. Type 2 diabetes mellitus patients should be screened and treated for traditional liver-related risk factors, including alcohol use disorders.

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Figure:

Attributable fractions for risks of liver disease progression among type-2 diabetes mellitus patients (N=26,569)
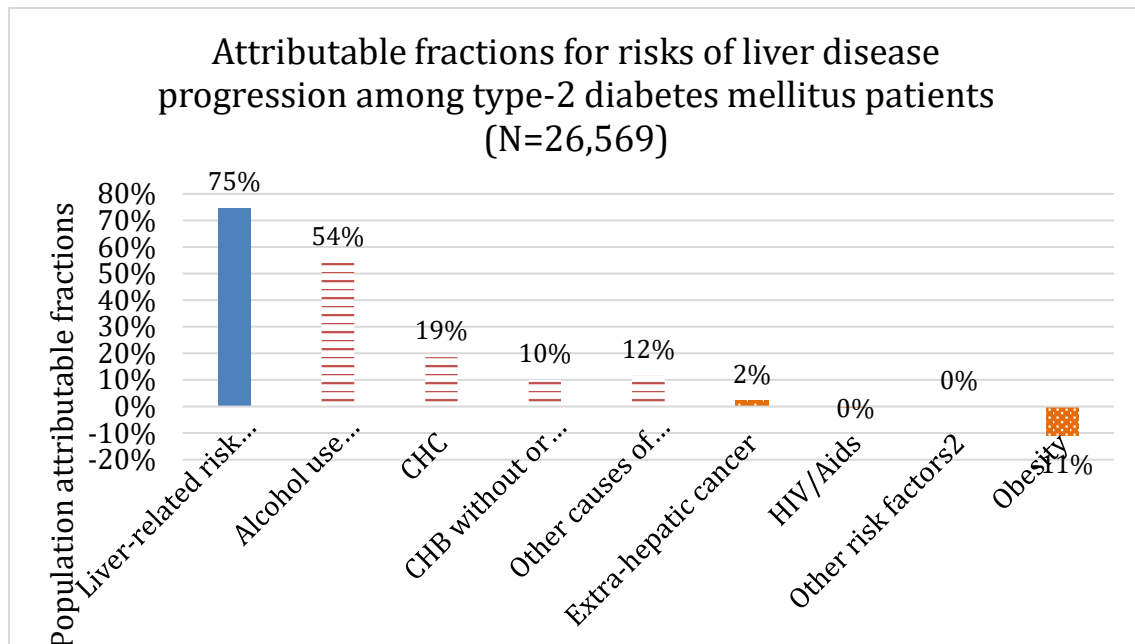
Note: CHC; chronic hepatitis C; CHB: chronic hepatitis B; CHD: chronic hepatitis D. Other risk factors include transplant recipients; connective tissue disorders.

P05-02YI Changes in individual free fatty acids during an oral glucose tolerance test in non-alcoholic fatty liver disease subjects stratified by body mass index

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Background and aims: Increased free fatty acid (FFA) plasma concentrations and adipose tissue insulin resistance (adipo-IR) are characteristic features of subjects with non-alcoholic fatty liver disease (NAFLD). Saturated fatty acids, either from diet or *de novo* lipogenesis, have been shown to be harmful to the liver and to be associated with increased risk of cardiometabolic diseases including NAFLD. While several studies have reported increased FFA levels during fasting and decreased suppression of lipolysis by insulin, information on FFA composition are less reported.

We aimed to evaluate FFA concentration and composition in NAFLD subjects during fasting and their insulin-mediated changes during an oral glucose tolerance test (OGTT).

Method: We recruited 29 NAFLD subjects and stratified them by body mass index (BMI) into non-obese NAFLD-BMI<30 (n = 11), obese NAFLD-BMI 30-45 (n = 10) and morbid obese NAFLD-BMI>45 (n = 8). Subjects underwent an OGTT with blood collected at baseline, 60, 120 and 180 min for measurement of glucose, insulin, and FFA concentration and composition (i.e., myristic, palmitoleic, palmitic, oleic, linoleic and stearic acid measured by gas chromatography/mass spectrometry). Insulin resistance (IR) was evaluated as HOMA-IR (Glucose*Ins/22.5), hepatic-IR (Hep-IR = EGP*Ins) and Adipo-IR (Lipo-IR = Ra_glycerol*Ins), where the endogenous glucose production (EGP) and lipolysis (Ra_glycerol) were measured by tracer infusion. Insulin sensitivity was calculated by OGIS.

Results: NAFLD subjects had similar glucose and insulin during the OGTT. Indexes of insulin resistance and liver stiffness did not differ between the study groups. Fasting FFA levels were not different between the groups, except for oleic acid which was lower in non-obese NAFLD in comparison with morbid obese NAFLD (p = 0.004). FFA composition during OGTT was significantly marked by a more efficient suppression of saturated fatty acids myristic, palmitic and stearic in morbid obese NAFLD in comparison with non-obese NAFLD. A mild but significant suppression of unsaturated fatty acids oleic and linoleic was observed between morbid obese NAFLD and non-obese NAFLD, but this is only seen during the first hour of the OGTT (t = 0-60 min).

Conclusion: During OGTT, the suppressive effect of insulin is more markedly seen in saturated fatty acids than in unsaturated fatty acids and this suppression is even higher for morbid obese NAFLD subjects.

P05-03 Early immunological modifications of the intestinal barrier in response to a non-alcoholic steatohepatitis-inducing diet

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Background and aims: Non-alcoholic steatohepatitis (NASH) is frequently observed in the metabolic syndrome. The gut-liver axis has been recognized to participate in the pathogenesis of numerous chronic liver diseases. A growing body of evidence highlights the importance of altered intestinal permeability, which can lead to lipopolysaccharide leakage into the portal vein and subsequent liver inflammation. We hypothesize that dysfunction of the intestinal barrier and its loss of integrity could initiate progression of fatty liver to NASH.

Method: Eight week old C57Bl6/J male mice were fed for one to four weeks with a high fat/high sucrose diet supplemented with cholesterol to induce histological NASH after 24 weeks, and compared to their littermate controls under chow diet. Immunophenotyping was performed on cells purified from the *lamina propria* of the small intestine by using a Percoll gradient after EDTA treatment and collagenase digestion. A 18 antibodies panel was used to identify immune cell subtypes by flow cytometry (Fortessa X20).

Results: After one week of diet, no change was observed in the lymphocyte ratios among immune cells in the *lamina propria* of the small intestine. However, after two weeks of the NASH inducing diet, changes in T lymphocyte subtypes were identified. Indeed, the number and also the proportion of TCRalpha/beta positive cells among T lymphocytes decreased. This decrease was mainly due to a reduction of CD8alpha/alpha positive cells, along with an increase of the CD8alpha/beta positive cells. After four weeks of diet, no changes in T lymphocyte fractions were observed, but a decrease of effector memory T (CD44+ CD62L-) and naive cells (CD44- CD62L-) in parallel to an increase of central memory T cells (CD44+ CD62L+) was observed.

Conclusion: A NASH inducing diet modifies the intestinal immunological barrier early after initiating the diet. While T lymphocyte ratios were normalized after four weeks, activation markers indicated enhanced recruitment of T lymphocytes. It will now be important to decipher the molecular mechanisms involved in the modifications of the intestinal immunological barrier induced by a NASH diet to test its critical role in the progression of the pathology.

Figure:

P05-04YI The efficacy of L-carnitine administration in non-alcoholic fatty liver disease patients

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Background and aims: Prevalence of non-alcoholic fatty liver disease (NAFLD) is increased in recent decades around the globe. Currently, the issue of NAFLD treatment is debatable. Carnitine is an organic compound involved in the transport of long-chain fatty acids from the cytoplasm to mitochondria, where their β-oxidation occurs, is considered among the possible medications that can favorably affect the treatment outcome in these patients. The aim of the study was to investigate the efficacy of L-carnitine administration to NAFLD patients.

Method: 60 NAFLD patients aged from 21 to 72 (54, 15 ± 12, 3) years were examined. The 1st group included 30 NAFLD patients, who in addition to the basic management were prescribed L-carnitine 2 g once daily intravenous for 14 days. The 2nd group involved 30 NAFLD patients who were recommended a standard management. The control group comprised 45 healthy individuals. Biochemical blood parameters, lipid profile, plasma levels of tumor necrosis factor-α (TNF-α), interleukin 10 (IL-10) and transforming growth factor-β1 (TGF-β1), leptin and adiponectin were investigated twice, first time before the investigation started and second after two weeks. Written informed consents were obtained from all the participants.

Results: Patients of the 1st group noted a reduction in aspartate aminotransferase activity by 51.5% (p = 0.03) and alanine aminotransferase activity by 50.9% (p = 0.046), that supports JC Bae et al. (2015) findings who reported about decreased cytolysis under Carnitine administration. Gamma-glutamyltransferase activity decreased in patients of both groups, more considerable in the 1st group-by 55.8 (p = 0.04), in the 2nd group-by 27.7 (p = 0.03). TNF-α in blood decreased after two weeks only in patients of the 1st group by 39.8% (p = 0.04). Il-10 and TGF-β1 plasma levels did not undergo significant changes during treatment in patients of both groups. Inspected patients were characterized by increased leptin and decreased adiponectin plasma levels indicating adipokine imbalance typical for NAFLD. Leptin blood level decreased by 44.1% (p = 0.02) and adiponectin concentration increased in 2.03 times (p = 0.03) after two weeks in patients of the 1st group which was not defined in those of the 2nd group.

Conclusion: Additional administration of L-carnitine in the management of NAFLD decreases cytolytic activity and proinflammatory TNF-α plasma level and improves adipokine imbalance indicating it as a promising medication in these patients.

Figure:

P05-05YI A deep learning algorithm to quantify liver fat content in humans

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Background and aims: Novel tools harnessing recent advancements in the field of artificial intelligence and digital pathology can be used to further our understanding in the pathophysiology and mechanisms of non-alcoholic fatty liver disease (NAFLD). To determine whether convolutional neural networks can be used to accurately quantify features of hepatic steatosis in histological images, we developed and validated a deep learning algorithm in large cohorts of human liver biopsies.

Method: We obtained liver biopsies from 668 bariatric surgery patients (mean age 48.6 ± 0.4 years, mean BMI 42.7 ± 0.3 kg/m^2). Herovici-stained liver specimens were digitized and whole-slide images were acquired. Liver histology was determined conventionally by three experienced liver pathologists. We used 107 liver biopsies and 2.9 Gpx of image data to train algorithm to recognize hepatic parenchyma, lipid droplets, portal areas, and capsular regions. The algorithm was validated against assessments by pathologists and other human observers. We also developed a steatosis grade classifier for automatically grading the degree of steatosis using the algorithm.

Results: The deep learning algorithm recognized individual lipid droplets with high sensitivity and precision compared to human counting ($r = 0.98$, 95% CI [0.96, 0.99]; Precision = 0.97; Recall = 0.90). The percentage of liver fat determined by the algorithm correlated highly significantly with pathologists' semi-quantitative assessments, being consistent for different pathologists and with wedge or needle biopsies ($r = 0.95$, 95% CI [0.93, 0.96]). The steatosis grade classifier reached higher machine-pathologist agreement ($\kappa = 0.67\text{-}0.73$) than any inter-pathologist concordance observed in this study ($\kappa = 0.56\text{-}0.72$). Pathologists' visual estimations were found to overestimate the degree of steatosis by 3.3 times on average. To characterize the acinar distribution of fat, we implemented a method to measure the distance of each individual fat droplet from the border of the nearest portal area (see figure).

Conclusion: We could accurately and rapidly analyse the percentage of macrovesicular steatosis, along with the number, size and localization of lipid droplets in human liver samples using the deep learning algorithm. These novel metrics can be used to further characterize histopathological features of the emerging subtypes of NAFLD.

Figure:
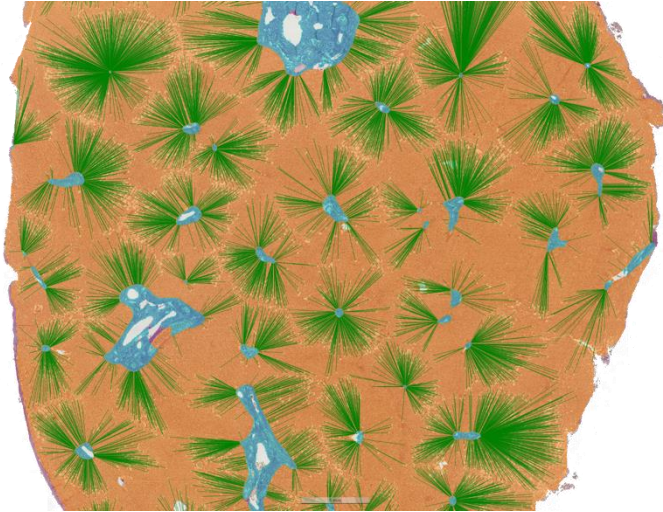
P05-06YI The effect of subclinical hypothyroidism on cardiovascular aging in individuals with non-alcoholic fatty liver disease

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Background and aims: The early detection of cardiometabolic risk factors, such as dyslipidemia, insulin resistance, markers of inflammation and vascular aging, has an important role in the prevention of cardiovascular complications. This is especially true for people with non-alcoholic fatty liver disease (NAFLD), which is associated with insulin resistance and frequently occurs with features of the metabolic syndrome. Given the prevalence and late detection of subclinical hypothyroidism (SH) among the population, it seems interesting to study its effect on the cardiovascular aging processes in patients with NAFLD.

Objective: To evaluate cardiometabolic changes and telomere length depending on the age in patients with NAFLD combined with SH compared to patients with NAFLD only.

Methods: Our study included 178 patients: 70 men (39.3%) and 108 women (60.7%), with verified NAFLD. Patients were divided into two clinical groups: 1 group (n = 75)-patients with NAFLD, their mean age was 47.2 ± 2.6 years, 2 group (n = 103) patients with NAFLD in combination with SH, the mean age was 56, 8 ± 6, 5 years. Clinical, biochemical, immunoassay and ultrasound parameters were assessed.

Results: Patients with NAFLD and SH had pro-atherogenic lipid profile and significantly greater values of total cholesterol ($p = 0.015$), very-low-density lipoprotein cholesterol ($p = 0.003$) in comparison to patients with NAFLD. Changes of the vascular endothelial growth factor ($p = 0.015$) and C-reactive protein ($p = 0.000$) values also were more pronounced in patients with concomitant SH. Insulin resistance based on fasting glucose ($p = 0.000$) and insulin ($p = 0.000$) levels was lower in the group with NAFLD only. It was found that manifestations of endothelial dysfunction and the process of age-dependent shortening of the telomere length in the buccal epithelium were more evident at the age of over 50 years among 2 group ($p = 0.000$).

Conclusion: Presence of SH determines the progression rate of vascular aging in patients with NAFLD, worsening cardiometabolic parameters in varying degrees depending on the patient's age.

P05-07 Omitted liver disease screening in acute screening coronary syndrome hides prevalence of high levels of severe liver fibrosis

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Background: The link between cardiovascular disease (CVD) and metabolic syndrome (MS) is known and metabolic parameters are assayed in CVD patients. Since non-alcoholic fatty liver disease (NAFLD) is also closely associated with MS, NAFLD remains surprisingly unexplored in patients with CVD. Recent studies have shown that the presence of NAFLD significantly influences the course of CVD (1, 2, 3). Also, with the advent of novel drugs treating NAFLD, case finding becomes more important. Aim: To determine the presence and severity of potential NAFLD in patients admitted with acute coronary syndrome (ACS).

Method: We retrospectively reviewed the admissions of all patients presenting with ACS to a major London Teaching Hospital, Guy's and St Thomas', in a 12-month period. We examined whether a full liver screen (FLS) was performed to allow calculation of a fibrosis score (FIB-4). As a secondary outcome we reviewed metabolic diagnoses for each patient.

Results: In the 12months to December 2018, 521 patients presented with ACS: 360 (69%) met the inclusion criteria of having ACS and surviving until discharge: 272 males, 88 females, aged 26-99 years old. The majority, 181 (50.3%), had an ST elevation Myocardial infarction, 176 (48.7%) a Non-ST elevation Myocardial Infarction and 3 (0.8%) an unspecified ACS. Only 30 patients (8.3%) had a full liver screen including aspartate transaminase to allow calculation of a FIB-4. 263 patients (72%) had basic liver function tests performed, and 67 (19%) had no LFTs measured at all. In those 30 patients with a full liver set, 47% had a FIB-4 score >3.25, indicating a very high extent of fibrosis, bridging fibrosis or cirrhosis (4). Only 2 patients from the data set had a pre-existing diagnosis of NAFLD.

Conclusion: Very few patients presenting with ACS are being assessed for liver fibrosis. In those assessed here, a significant proportion had advanced liver fibrosis. A change in practice to perform a FLS on patients admitted with ACS would allow for the increased diagnosis of liver fibrosis and would enable earlier management to reduce morbidity.

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P05-08YI Obese patients carrying NAFLD-associated genetic variants present specific serum and liver lipidomic profiles: identification of a lipidomic signature in serum to estimate the liver fat content

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Background and aims: Novel serum-derived metabolomic tests were generated to diagnose NAFL and NASH in obese patients. Here, we investigated: 1) whether obese individuals harboring the *PNPLA3* p.I148M, *TM6SF2* p.E167K, and *MBOAT7* p.G17E variants, associated with increased risk of steatosis and fibrosis, present specific lipidomic profiles in both serum and liver, and 2) the potential of particular lipidomic signatures to estimate the liver fat content.

Method: Hepatic steatosis was determined by magnetic resonance imaging (MRI fat fraction), and by histopathology of liver tissue from obese individuals (n = 114; BMI>35kg/m^2). Serum lipidomic profile was analyzed by UPLC-MS and a specific signature was correlated with the liver fat content. In parallel, 225 obese patients were genotyped the *PNPLA3* p.I148M, *TM6SF2* p.E167K, and *MBOAT7* p.G17E variants using allelic discrimination TaqMan assays. Serum (n = 225) and liver (n = 53) lipidomic profiles were measured.

Results: The *PNPLA3* p.I148M, *TM6SF2* p.E167K, and *MBOAT7* p.G17E variants were found in 42%, 10% and 72% patients, respectively. Patients harboring the *PNPLA3* p.I148M variant (in hetero- or homozygosity) were characterized by reduced levels of certain triglycerides ($p < 0.05$) in serum, while liver presented an accumulation of multiple di- and triglycerides (at least $p < 0.05$). Patients with the *TM6SF2* p.E167K variant showed decreased levels of certain ceramides, di- and triglycerides in serum compared to WT patients (at least $p < 0.05$). In addition, circulating glycerophospholipids, ceramides, and certain FA were decreased in patients with the *MBOAT7* p.G17E variant compared to WT patients (at least $p < 0.05$). Patients harboring the 3 variants (in hetero- or homozygosis) presented a completely altered lipidomic profile in serum compared to obese controls, namely a decrease in di-, triglycerides and saturated, mono- and polyunsaturated FA (at least $p < 0.01$). On the other hand, we identified 11 lipids in serum that, within a new algorithm, correlated with MRI fat fraction (r = 0.815; r^2 = 0.664; $p < 0.001$), the grade of steatosis and NAS score measured by histopathology.

Conclusion: Obese patients harboring genetic risk variants for NAFLD/NASH are characterized by specific lipidomic profiles, which may participate in disease pathogenesis and represent new tools to estimate prognosis. We also describe a novel lipidomic signature in serum that allows to estimate fat content in the liver of obese patients, embodying an innovative tool to monitor fat accumulation.

P05-09YI lncRNA-H19 as an epigenetic biomarker of liver cancer stem cells

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Background and aims: Epigenetics play an important role in the progression of non-alcoholic fatty liver disease (NAFLD) to hepatocellular carcinoma (HCC). Liver cancer stem cells (LCSCs) could be responsible for the carcinogenesis, recurrence, metastasis and chemoresistance of HCC.
The aims of our study were: a) to isolate and characterize LCSC and to analyse the epigenetic profile; b) to confirm these findings in an animal model of NAFLD-HCC fed with high fat diet supplemented with cholesterol and fructose.

Method: *In vitro*, EpCAM and CD133 positive cells were isolated from Huh7.5 cell line by FACS (Facs Aria Fusion BD). Epigenetic and transcriptomic profile were studied by ClarionD and miRNA4.0 arrays and candidates' gene by qPCR (*NANOG, SOX2, OCT4* and *H19*).
Eleven male 6-weeks old mice C57BL/6J were fed a HFHCC diet (40% Kcal fat, 1% cholesterol and 42g/L glucose/fructose in drinking water) (n = 9) or standard diet (n = 2) for 52 weeks. Anatomorphological, histological, biochemical and metabolic changes were measured.

Results: An increase in size and number of spheroids were observed in EpCAM+CD133+ cells (fold-sphere number: 2.15 ± 0.96; $p = 0.004$ and fold-size µm2: 3.04 ± 1.93; $p < 0.001$ compared to Huh7.5).
LCSC showed higher expression of *NANOG* (fold-2.66 ± 0.38; $p < 0.0001$), *SOX2* (fold-3.52 ± 0.62; $p = 0.063$), *OCT4* (fold-1.94 ± 0.17; $p < 0.01$), *ABC-T* (fold-2.36 ± 0.6; $p < 0.05$) and lncRNA-*H19* levels (fold-2.18 ± 0.32; $p = 0.003$).
HFHCC-diet-induced NASH in mice, was characterized by macro-microvesicular steatosis, inflammatory foci at lobular, portal and periductal levels, followed by presence of hepatocyte degeneration (ballooning) and moderate fibrosis. Besides, we also detected nodules in 33.3%, which were classified into adenomas or well-differentiated HCC. *Gpc3+* expression was significantly raised in HCC in comparison with animals with NASH without HCC and healthy animal controls. LncRNA-*H19* were found up-regulated in HCC liver tissue compared to NASH and control ($p = 0.007$)

Conclusion: LncRNA-*H19* was found increased in HCC liver tissue from NAFLD animal model as well as in EpCAM+CD133+ liver cancer stem cells. More studies are needed in order to clarify the role of lncRNA-*H19* in HCC development.
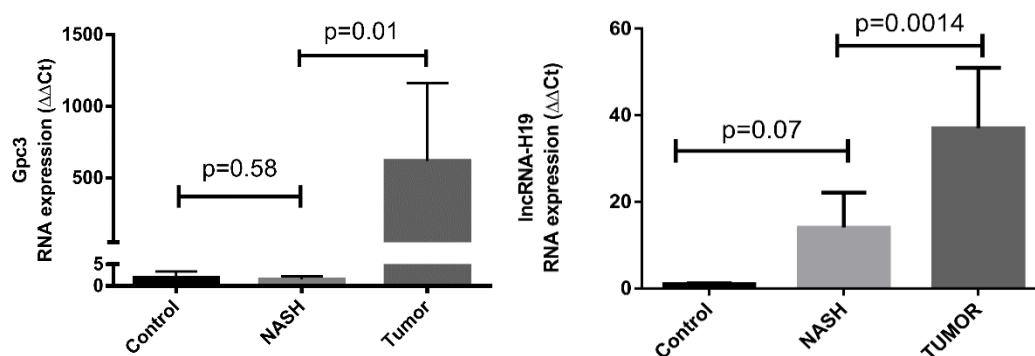
Figure:

P05-10YI NAFLD population in Northern and Southern Italy. A longitudinal and epidemiological study in four tertiary centers

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Background and aim: NAFLD is becoming the leading aetiology of liver disease in Europe. However, its presentation may vary according to the European region, even in the same country. Our aim was to compare NAFLD patient features in four tertiary centres in Northern Italy (NI) and Southern Italy (SI).

Methods: 853 Caucasian patients underwent a liver biopsy for suspicion of NAFLD in main tertiary centres in Italy (Turin, Milan, Rome and Palermo). Clinical and biochemical data were collected at the time of biopsy. Patients have then undertaken regular visits for routine care and clinical events were recorded by clinicians.

Results: patients from NI and SI were 454 and 399 respectively. Females were 25% (n = 114) and 29% (n = 114) in NI and SI respectively (p = ns) and mean age was 45, 8 in NI and 44, 2 in SI (p = ns). Mean BMI for NI and SI was 27, 5 and 29 respectively (p < 0, 001) and lean patients (BMI <25) were 29, 3% (n = 132) in NI and 18, 3% (n = 73) in SI (p < 0, 001). The prevalence of advanced fibrosis in NI was 20% (n = 91), while in SI it was 15% (n = 60) (p = 0, 04); NASH prevalence was 52, 8% (n = 238) and 83, 7% (n = 334) in NI and SI respectively (p < 0, 001) and patients with steatosis without fibrosis (F0) were 173 in NI (38, 3%) and 64 in SI (16%) (p < 0, 001). Diabetes prevalence in NI patients was 19, 5% (n = 88) while it was 16, 5% in the SI cohort (n = 66) (p = ns). PNPLA3 C>G polymorphism (either homozygous or heterozygous) was 68, 1% in NI (152/223) and 88, 1% in SI (170/193) (p < 0, 001). After a median follow-up of 84 months, no significant differences were found in the incidence of cardiovascular events or HCC; conversely, a higher incidence of hepatic decompensation was reported in the NI cohort [OR 3, 9 (1, 9-7, 6), p < 0, 001], and advanced fibrosis was the only independent predictor at the multivariate analysis [OR 3, 4 (1, 6-7, 2)]. The survival analysis did not demonstrate a significantly different prognosis between NI and SI patients and advanced fibrosis was the main independent predictor of death in both cohorts [OR 6, 3 (2-19, 6) p = 0, 002].

Conclusion: Although patients in SI have a higher BMI, a higher prevalence of NASH, consistent with a higher prevalence of PNPLA3 C>G mutation, advanced fibrosis is more prevalent in Northern Italy, associated with a higher rate of liver disease progression. Being advanced fibrosis a predictor of mortality in both cohorts, this study confirms the need of research and clinical focusing on fibrosis, which may also occur at lower BMI values.

P05-11YI Differences in anxiety and depressive symptomatology of NAFLD/NASH patients according to fibrosis stage

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Background and aims: There is evidence of the association of psychopathological comorbidity in NAFLD/NASH and the presence of advanced histological alterations. This study therefore proposed a comparison of anxiety and depressive symptomatology in patients with biopsy-proven NAFLD and NASH depending on whether or not they had significant fibrosis.

Method: On the one hand, a group of 175 NAFLD patients (111 men and 64 women) without significant fibrosis (F0-F1) (G_1), aged 53.07 ($SD = 11.66$) was selected. On the other, a group of 291 NASH patients made up of 163 men and 128 women, aged 55.49 ($SD = 11.81$) was also selected. This group was divided into two subgroups: 159 patients with significant fibrosis ($\geq$F2) (G_2) and 132 without significant fibrosis (G_3), classified according to liver biopsy. All of them were evaluated using the following instruments: psychosocial interview, Hospital Anxiety and Depression Scale (HADS) and Beck Depression Inventory-II (BDI-II). To compare the anxiety-depressive symptomatology between groups, the Welch's U was computed as an omnibus test, and for post hoc multiple comparisons, Games-Howell, and Bonferroni corrected Mann-Whitney's U tests ($0.05/2 = 0.025$) were applied. Cohen´s d was used as an index of effect size.

Results: Statistically significant differences were found in the three variables analyzed: anxiety ($p = 0.004$) and depression ($p = 0.000$) measured with the HADS, and depression ($p = 0.000$) measured with BDI-II. The most relevant differences (medium effect size) were that G_2 had higher scores in depressive symptomatology than G_1 ($p = 0.000$, $d = -0.531$, HADS; $p = 0.000$, $d = -0.501$, BDI-II) or G_3 ($p = 0.000$, $d = 0.573$, HADS; $p = 0.000$, $d = 0.628$, BDI-II). Specifically, the items with the most statistical weight in these differences, because of their relevant effect sizes were: "I feel as if I am slowed down" (G_1-G_2: $p = 0.000$, $d = -0.546$; G_2-G_3: $p = 0.000$, $d = 0.567$), "worthlessness" (G_2-G_3: $p = 0.000$, $d = 0.525$), "loss of energy" (G_1-G_2: $p = 0.000$, $d = -0.603$; G_2-G_3: $p = 0.000$, $d = 0.559$), and "tiredness or fatigue" (G_1-G_2: $p = 0.000$, $d = -0.598$; G_2-G_3: $p = 0.000$, $d = 0.587$).

Conclusion: NASH patients with significant fibrosis show greater depressive symptomatology, that is, poorer mental health than those with NAFLD and NASH without significant fibrosis.

P05-12 Nover 3D Human NASH model for high-throughput compatible efficacy testing

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is characterized by excessive accumulation of lipids within the liver and can progress from simple fatty liver to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis. Currently, there is no approved, safe therapy for NASH and the development of novel therapeutics is impeded by the lack of predictive *in vitro* models to understand the complex mechanisms underlying disease initiation and progression. The aim of the study was to develop a 3D Human NASH model for high-throughput compatible efficacy testing.

Method: We developed a protocol for induction of NASH with free fatty acids and LPS in medium containing high levels of sugars to recapitulate NASH pathogenesis and for drug efficacy testing *in vitro* and analysed characteristic markers like lipid loading using triglyceride assays, secretion of proinflammatory markers (Luminex) and fibrosis (pro-collagen type I secretion).

Results: Here, we present a human *in vitro* 3D cell model containing primary hepatocytes, Kupffer cells, endothelial cells and hepatic stellate cells. Upon treatment with lipotoxic stimuli free fatty acids and LPS these microtissues showed key physiological aspects of NASH. The lipotoxic stimuli promoted lipid accumulation within hepatocytes and increased tissue triglyceride levels. These changes were accompanied by increased inflammatory and pro-fibrotic markers secretion. Consistent with clinical trial data, Elafibranor, an PPARα/β agonist decreased tissue lipid accumulation and triglycerides levels. Additionally, Elafibranor down-regulated the secretion of inflammatory markers (TNF-α, IL-6, IL-8, MCP-1, MIP-1α and IP-10) and pro-collagen type I. These data overlapped well with the clinical findings demonstrating anti-steatotic, anti-inflammatory and anti-fibrotic effects of Elafibranor. Testing anti-NASH compounds currently in clinical drug development will demonstrate the relevance of the model to the clinical situation.

Conclusion: In summary, using this *in vitro* system to test novel drug candidates prior to clinical trials represents a promising approach for the early selection and decision making of the most effective and less toxic compounds to move further in the drug development process.

P05-13YI Elevated expressions of Sodium taurocholate co-transporting polypeptide (NTCP) on NK cells impaired their function and contribute to liver fibrosis in NAFLD patients

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Background and aim: Hepatic nuclear receptors are known to modulate genes controlling bile acids (BA) metabolism, thus, we aimed to evaluate BA transporter-sodium+/taurocholate co-transporting polypeptide (NTCP) on NK cells as a modulator in NAFLD progressions.

Method: Twenty-four patients biopsy-proven NALFD were classified according to the NAFLD activity score. Liver biopsies and peripheral NK-cells were evaluated for NTCP expressions by the confocal microscopy and western blot/flow cytometry. Modulations of NTCP expressions was made through Epigallocatechin 3-Gallate (EGCG) (NTCP antagonist) and HBsAg (NTCP agonist).

Results: NTCP expressed on liver NK-cells of patient's biopsies and NK peripheral-blood of healthy and NASH patients. These results were associated with alterations in NK cells activity; NK-cells stimulation marker (CD107a) over-expressed in low-fibrosis patients, but kept low in advanced-fibrosis (*NK impairment*). NTCP expressed on $37.6 \pm 8\%$ of healthy peripheral NK-cells, and significantly increased gradually in NAFLD fibrotic stages ($p < 0.05$). EGCG inhibited NTCP on NK livers and were associated with amelioration in their function. HBsAg while did not modulate NTCP, it inhibited Granzyme-b and deactivated NK activity. NK-cells form NAFLD patients with F4 fibrosis showed reduction in PI3K and ERK/MAP kinase pathways correlated with inhibitions in mTOR activity ($p = 0.001$).

Conclusion: BA-activated NTCP decreased NK activity signalling pathway through PI3K and mTOR inhibitions indicating inverse association between and NTCP and NK activity. Expressions of NTCP in NK-cells (known as anti-fibrotic cells) are associated with progression of NAFLD and suggest their role in liver fibrosis. Modulatory therapies targeting BA-transporter NTCP and NK- cells could prevent complications to liver cirrhosis.

P05-14 Combined treatment with L-carnitine and nicotinamide riboside improves hepatic metabolism and attenuates obesity, hepatic peroxidation and steatosis

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Background and aims: Obesity characterized by adiposity and ectopic fat accumulation is associated with the development of non-alcoholic fatty liver disease (NAFLD). Treatments that stimulate lipid utilization may prevent the development of obesity and comorbidities. This study evaluated the potential anti-obesogenic and hepatoprotective effects of combined treatment with L-carnitine and nicotinamide riboside, i.e. components which can enhance fatty acid transfer across the inner mitochondrial membrane and increase NAD+ levels necessary for β-oxidation and TCA cycle, respectively.

Method: L-carnitine (LC; 0.4% w/w), nicotinamide riboside (NR; 0.3% w/w) and a combination of both (COMBI) were supplemented to a high fat diet (HFD). Ldlr-/-.Leiden mice, a preclinical model with translational obesity and NAFLD characteristics, were fed these diets including chow and HFD for 21 weeks to evaluate their effects on general obesity, hepatic steatosis and lipid peroxidation (4-hydroxynonenal adducts). EchoMRI was used to study body composition during the study. Plasma metabolomics, histology and genome-wide liver transcriptomics were analyzed after 21 weeks.

Results: L-carnitine plasma levels were reduced by HFD and normalized by LC. NR supplementation raised its plasma metabolite levels demonstrating effective delivery. Although food intake and ambulatory activity were comparable in all groups, COMBI treatment significantly attenuated HFD-induced body weight gain, fat mass gain (-17%) and hepatic steatosis (-22%). Also, NR and COMBI reduced hepatic lipid peroxidation. Upstream-regulator gene analysis demonstrated that COMBI reversed detrimental effects of HFD on liver metabolism pathways and associated regulators, e.g. ACOX, SCAP, SREBF, PPARGC1B, and INSR.

Conclusion: Combination treatment with LC and NR exerts protective effects on lipid peroxidation, metabolic pathways and constitutes a new approach to attenuate HFD-induced obesity and NAFLD.

P05-15YI acNASH Index: a novel screening tool for non-alcoholic steatohepatitis patients with persistent normal alanine aminotransferase

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Background and aims: Current non-invasive methods could not meet the need to screen the vastly underdiagnosed NASH patients with normal alanine aminotransferase levels (nALT). We aim to construct one simple index to diagnose NASH patients with persistent nALT.

Method: Consecutively, 80 biopsy-proven NAFLD for the training group and 53 independent biopsies for validation group were included in this study. Persistent nALT was defined as ALT<40 U/L for 3 months, while NASH was defined as NAS ≥5. Univariate and multivariate analysis were utilized to screen diagnostic variables and subsequently used to develop a diagnostic index for NASH from the training group. Area under receiver operating characteristics (AUROC) of formulated index was compared with HAIR score, NICE model and liver apoptotic biomarker CK-18 M30.

Results: Serum aspartate aminotransferase (AST) and creatinine (Cr) were independently associated with nALT NASH, and thus were selected for constructing the acNASH index. For the training group, acNASH index obtained an ideal AUROC of 0.826, while HAIR score, NICE model and CK-18 M30 had subpar AUROC of 0.531, 0.582 and 0.544, respectively, ($p = 0.003$, $p = 0.004$, $p = 0.004$; respectively). The diagnostic performance of acNASH index was well-established by the validation group with AUROC of 0.786, while HAIR score was 0.526 ($p = 0.030$).

Conclusion: With validation, acNASH index, comprised of routine laboratory variables, demonstrated promising discriminatory ability in persistent nALT NASH compared to existing non-invasive methods. It could be implemented and serve as a tool to screen or diagnose nALT NASH in primary care prior to referral.

Figure:
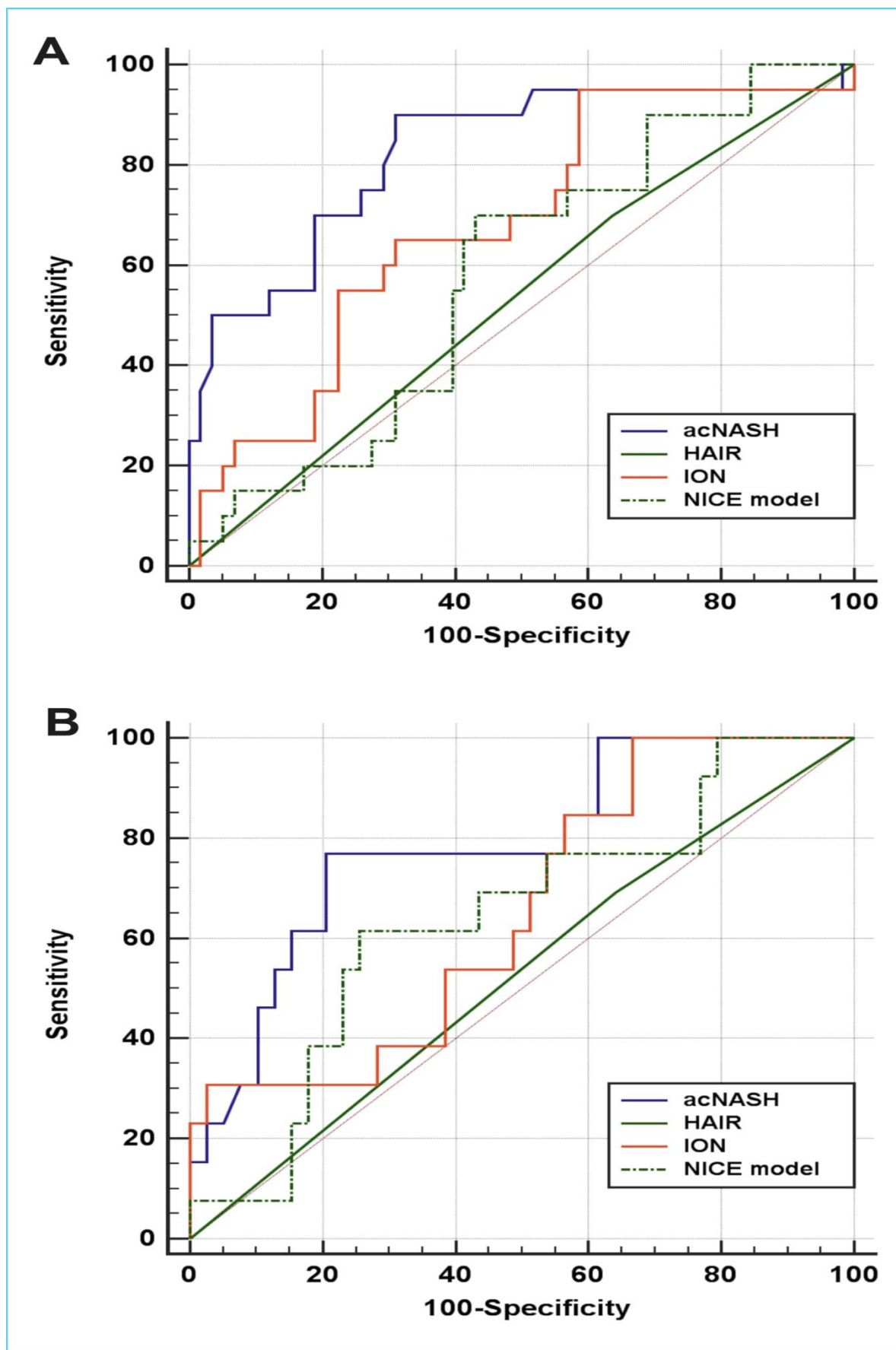
P05-16 PNPLA3 could help the clinicians to individuate NAFLD subjects at major risk of disease progression: a single center cohort study

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Background and aims: NAFLD is rapidly increasing worldwide, but only a minority of patients (pts) shows a progressive liver disease (LD). Genome-wide association studies showed that single-nucleotide polymorphisms (SNPs) in PNPLA3 e TMSF2 are associated with LD severity. We investigated the correlation of these SNPs and the LD stage in NAFLD pts

Method: 240 consecutive NAFLD pts (M:F = 148:92, 53 ± 16, 84 yrs) underwent clinical evaluation including: AST, ALT, GGT, ALP, blood count, p.glucose, insulin, HOMA, total-cholesterol, LDL, HDL, triglycerides, ferritin; body mass index (BMI) and waist circumference; liver stiffness by transient Elastography (TE, Fibroscan®), Fibrosis 4-score (FIB-4) and NAFLD fibrosis score; liver steatosis by controlled attenuation parameter (CAP), fatty liver index (FLI) and hepatic steatosis index (HSI); SNPs of PNPLA3 (rs738409 C>G) and TM6SF2 (rs58542926 C>T). Non parametric tests were applied for statistical analysis

Results: PNPLA3: wild type (WT) variant was present in 95 pts (39.6%); heterozygosis (HE) in 98 (40.8%) and homozygosis (HO) in 47 pts (19.6%). *TMSF2:* WT in 205 pts (85.4%); HT in 31 (12.9%) and HO in 4 pts (1.7%). 2 pts (0.8%) were HO for both PNPLA3 and TMSF2. Overall HO for PNPLA3 vs WT/HE genotype showed a significant increase of TE [6, 7 (3.4-50) vs 5, 6 (2.7-64) kPa, P = 0.02], AST [32 (16-171) vs 26 (12-124) U/L, P = 0.08], ALT [44 (10-260) Vs 34 (9-222) U/L, P = 0.02], FIB-4 [1.26 (0.36-5) Vs 1.20 (0.30-6.99) P = 0.020] whilst lower values of total-cholesterol [177 (109-333) vs 190 (112-325) mg/dl, P = 0.05]. Significant fibrosis (TE >10 kPa) was present in 11/47 (25.7%) pts HO for PNPLA3 vs 22/192 (11.4%) WT/HT pts (p = 0.033). Pts<52 years (47, 1%), HO for PNPLA3, had higher TE [6, 3 (4-12.1) vs 5, 4 (2, 7-46.4) kPa, P = 0.04]; pts>52 years (52, 9%) HO for PNPLA3 showed higher levels of TE [7, 6 (3, 4-50) vs 5, 8 (3-64) kPa, P = 0.020], AST [34 (20-82) Vs 26 (12-124) U/L, P = 0.002), ALT [43 (20-84) Vs 29 (9-132) U/L, P = 0.001) and lower values of PLTs [183 (109-124) Vs 222 (71-329) P = 0.028). No significant differences were found between HO vs WT/HT pts for TMSF2. For both SNPs no difference between HO and WT/HT emerged for CAP, HSI, FLI, prevalence of diabetes, hypertension, cardiovascular and malignant disease

Conclusion: In our cohort of NAFLD patients PNPLA3 screening proved useful to identify NAFLD patients at higher risk of LD progression. TMSF2 screening, due the lower prevalence, could be considered in selected cases

P05-17 IDL-2965: A selective, highly potent, clinical stage integrin antagonist for the treatment of NASH

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Background and aims: Clinical outcome in NASH is associated with the stage of liver fibrosis. RGD-binding integrins are attractive therapeutic targets for the treatment of fibrosis. IDL-2965 is a potent, small molecule integrin antagonist that is currently being characterized in a Phase 1/1b clinical program including healthy subjects, NASH patients and IPF patients.
Method: A robust drug discovery campaign identified integrin antagonists with strong antifibrotic activity and favourable PK. IDL-2965 emerged from these screens and was characterized for potency, selectivity, and antifibrotic efficacy, as well as for safety in a formal toxicology program.
Results: In cell-based ligand displacement assays, IDL-2965 displayed IC_{50} values of 1.6, 1.4 and 0.3 nM against $\alpha v \beta 1$, $\alpha v \beta 3$ and $\alpha v \beta 6$, respectively. In a cell-based co-culture system of TGF-β activation, IDL-2965 displayed an EC_{50} of 110 nM. IDL-2965 was metabolically stable in hepatocytes and microsome fractions across species, including humans ($t_{1/2}$ >240 min). Following oral dosing in rat and primate, IDL-2965 displayed an apparent $t_{1/2}$ of 7.4 hr and 8.5 hr, respectively. In rat, ^{14}C-IDL-2965 was eliminated by both hepatic and renal routes. In animal models, both prophylactic and therapeutic IDL-2965 treatment regimens markedly reduced liver fibrosis and fibrosis-related plasma biomarkers. Minimum effective doses in each model were ≤3 mg/kg. In a rat CCl_4 model, prophylactic once-daily oral treatment with IDL-2965 significantly reduced liver fibrosis (via histopathology). In a mouse CDAHFD model, once-daily oral therapeutic treatment with IDL-2965 significantly reduced fibrosis scores, liver hydroxyproline, and plasma CK-18 at 3 mg/kg. In a DIO-NASH model in ob/ob mice, therapeutic treatment with IDL-2965 reduced collagen and αSMA relative to baseline, and reduced plasma CK-18 and hyaluronic acid relative to vehicle treated animals. An improvement in NAFLD score was driven by a reduction in hepatocyte ballooning, with no impact on steatosis or inflammation. Pharmacodynamic studies in the DIO-NASH model further demonstrated that IDL-2965 reduced plasma CK-18 and TIMP-1 within one week, and reduced circulating hyaluronic acid by week 4. IDL-2965 displayed a favourable safety profile in formal toxicology studies.
Conclusion: IDL-2965 potently suppresses fibrosis and related biomarkers in preclinical models of liver fibrosis and its characterization in an ongoing Phase 1 program including NASH patients is warranted.

P05-18YI NEDDylation inhibition as a new potential therapy for Non-alcoholic Fatty Liver Disease

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Background and Aims: NAFLD comprehends a group of complex and chronic liver disease conditions with a 25% prevalence worldwide. The study of post-translational modifications (PTMs) has emerged as a faster and effective mechanism to regulate signaling pathways. NEDDylation is an ubiquitin-like reversible PTM characterized by the conjugation of NEDD8 (neural precursor cell expressed developmentally down-regulated 8) to target proteins promoting their stabilization. Our laboratory has previously described an increase of the liver NEDDylated proteome in hepatocellular carcinoma (HCC) as well as in patients and mouse models with liver fibrosis. Consequently, we decided to study the role of NEDDylation in the pathogenesis and progression of NAFLD as well as the potential use of an inhibitor of NEDDylation called Pevonedistat (MLN4924) in NAFLD therapy.

Method: Animal procedure: male adult C57BL/6 mice (3-month old) fed either with methionine (0.1%) and choline (0%) deficient diet (0.1%MCD diet) or with a choline-deficient high fat diet (CD-HFD) were used. After 2 week of feeding with 0.1%MCD diet or 3 weeks of the CD-HFD, a group of mice were treated during 2 or 3 more weeks, depending on the diet, with Pevonedistat (60mg/Kg) by oral gavage each 4 days. Proteomic analysis: liver samples were digested with trypsin and analyzed by LC-MS/MS using a TIMS Tof Pro powered by PASEF (Bruker). The differential expression analysis was carried out using PEAKS software (Bioinformatics Solutions Inc). Only proteins quantified with at least 2 different peptides identified with a FDR<1% were considered for further analysis. Functional analysis of the differential proteins (p < 0.05, ratio>1.5) was carried out using the DAVID Functional and Ingenuity Pathway Analysis (IPA, QIAGEN).

Results: Overall, the global NEDDylated proteome increased in liver and serum of NAFLD patients and mouse models. Moreover, hepatic gene expression levels of proteins involved in the NEDDylation pathway were modulate in NASH human and mouse models. In preclinical studies of mice fed with 0.1%MCD or the CD-HFD and treated with Pevonedistat, steatosis, inflammation and fibrosis were reduced in association with a reversion of the NEDDylation levels both in serum and in liver. Increased fatty acid oxidation (FAO) as well as decreased reactive oxygen species (ROS) levels were observed in 0.1%MCD or CD-HFD Pevonedistat-treated mice. Reduced ROS after NEDDylation inhibition is most probably a result of increased catalase activity and reduction of lipid peroxidation. In summary, NEDDylation inhibition is able to ameliorate NASH by increasing FAO and thereby improving liver steatosis by reducing ROS and inflammation.

Conclusion: NEDDylation plays an important role in the physiopathology and progression of NAFLD and a better understanding of the complexity between NEDDylation and NASH will pave the way to develop a novel therapeutic option.

P05-19 Predictors of high shear wave elastography (SWE) measurements among non-alcoholic fatty liver disease (NAFLD) patients in primary care

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Background and aims: Non-alcoholic fatty liver disease is prevalent in the general population. Previous studies have reported predictors of high liver stiffness measurements (LSM), including age or having elevated liver enzymes, in patients referred to tertiary care. However, these predictors have not been validated in primary care. Therefore, we aimed to identify patient characteristics and independent predictors of elevated LSM among NAFLD patients in the primary care setting, using data from the largest NAFLD clinical primary care pathway in North America.

Method: We used data from the Calgary NAFLD clinical care pathway (CNCCP) between March and November 2018. CNCCP has been developed by hepatologists, primary care physicians (PCP), and a multicenter community-based radiology group providing 2-dimensional shearwave elastography (2D-SWE) in Calgary, Alberta (population ~1.4 million). The CNCCP has enabled PCP to directly investigate NAFLD patients with 2D-SWE. Patients with any of the following conditions: overweight, obesity, diabetes mellitus, elevated liver enzymes, or fatty liver detected by prior imaging were eligible to be included in the pathway. Patients with suspected NAFLD had routine serology to exclude other causes of chronic liver disease. After evaluation with 2D-SWE, NAFLD patients with 2D-SWE LSM $\geq$ 8.0kPa (or inconclusive result) were referred to hepatology, while those with SWE<8.0kPa were managed by PCP using a standardized management pathway. Logistic regression models were used to identify independent predictors of LSM values $\geq$8.0kPa by 2D-SWE. Regression models were adjusted for patient demographics, comorbidities, body mass index (BMI), and ALT at LSM assessment.

Results: Through the CNCCP, we evaluated 2, 081 patients with suspected NAFLD from March-November 2018. NAFLD prevalence by ultrasound was 94.1% (n = 1, 958) and varied by BMI (97.1% among BMI$\geq$ 30; 92.3% for BMI 25-30). NAFLD patients were more likely women (53.7%) with a median age of 55 (IQR: 45-63). Patients having impaired glucose tolerance (IGT), DM type 2, hypertension, and obesity were prevalent in our cohort (33.6%, 28.7%, 40.8%, and 62.9% respectively). Overall, median SWE was 4.4 kPa (IQR: 3.7-5.5 kPa). In this cohort, only 67 patients (3.4%) had LSM by 2D-SWE $\geq$ 8.0kPa. In our adjusted models, obesity (aOR 1.93: 1.01-3.75), DM type 2 (aOR 2.18: 1.11-4.29), and having hypertension (aOR 2.17: 1.18-3.96) were the only independent predictors of having SWE $\geq$ 8.0kPa. Age, sex, elevated ALT, and IGT were not independently associated with elevated LSM.

Conclusion: In this large primary care based NAFLD cohort, we report that DM type 2, hypertension, and obesity were independent predictors of elevated LSM, but elevated ALT was not (common reason for NAFLD hepatology referral). These findings may enable PCP to prioritize evaluation of NAFLD patients in primary care based on patient comorbidities.

P05-20 Establishment of an novel non-alcoholic steatohepatitis model using a high fat and cholesterol diet in young rabbits

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Background and aims: Several studies have reported metabolic disorder may increase the risk of non-alcoholic liver disease in pediatric populations. The aim of the present study was to establish a non-alcoholic steatohepatitis (ANSH) model using a high fat and cholesterol in young rabbits and to investigate whether and how the NASH progressing to liver cirrhosis in young rabbits.

Method: A total of 31 male young rabbits (6 weeks age) were randomly divided into 3 groups including fed a normal chow diet for 20 weeks, a high fat and cholesterol diet (HFCD) for 4weeks, 6 weeks, 8 weeks and 20 weeks, respectively. Plasma ALT, AST, TG, TC, LDL-c, HDL-c, and FFA were measured. At 4, 8 and 20 weeks the fast blood glucose was measured, and a glucose tolerance test was performed. The liver damage was evaluated using hematoxylin and eosin staining; and the liver fibrosis was evaluated using Sirius Red staining.

Results: Plasma levels of ALT, AST, TG, TC, LDL-c and FFA in HFCD groups were significantly higher as compared with those in the control group both at 4 weeks, 6 weeks, 8 weeks and 20 weeks groups (p <0.001). Histological analysis (Figure 1) represented that HFCD groups demonstrated marked differences in the hepatocyte steatosis compared with the control group. HFCD feeding for 4 weeks significantly resulted in liver fatty changes and slightly fibrotic collagen deposition mainly in the portal area with minimal inflammatory cell infiltration. Additional 2 weeks of HFCD feeding represented a significant hepatocyte steatosis with paracellular fibrosis and inflammatory cell infiltration. HFCD for 8 weeks and 20 weeks resulted in a more severe hepatocyte steatosis and an advanced liver fibrosis, especially at 20 weeks of HFCD all livers represented a liver cirrhosis (Figure 2). Meanwhile, the inflammation was recognized more significantly in HFCD 20 weeks animals. Hepatocyte ballooning was observed science HFCD 8 weeks and more severe in 20 weeks.

Conclusion: The result suggests that HFCD may result in a NASH progressing fast and significantly in young rabbit and finally present a liver cirrhosis. This model might be useful for the future studies on area of metabolic and NASH in pediatric populations.
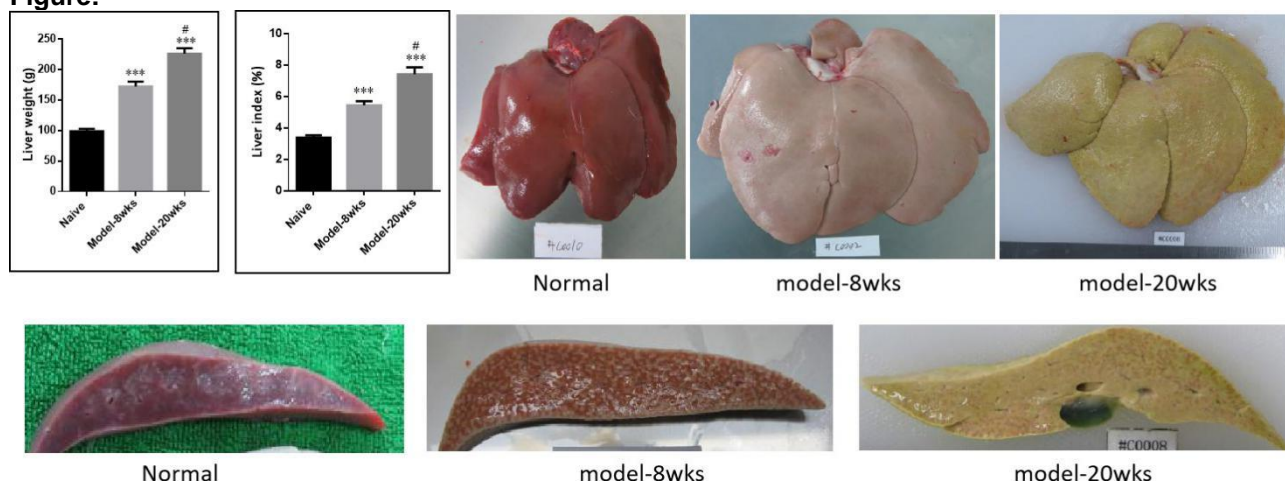
Figure:

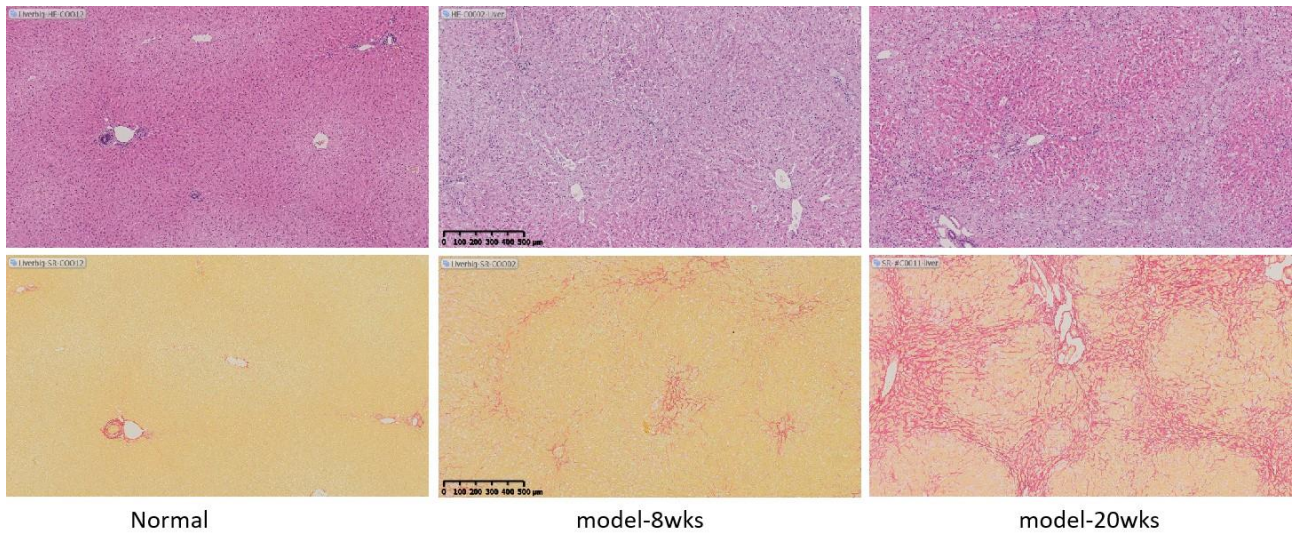Figure 1 Changes in liver morphology

Figure 2 Changes in liver histology

P05-21 Positive Results from REGENERATE: A Phase 3 International, Randomized, Placebo-Controlled Study Evaluating Obeticholic Acid Treatment for NASH

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Background and aims: Obeticholic acid (OCA), an FXR agonist, improved both fibrosis and histologic features of non-alcoholic steatohepatitis (NASH) in the Ph2 FLINT study. This Month 18 pre-specified interim analysis of the Ph3 REGENERATE study evaluated the effect of OCA on liver histology in patients (pts) with biopsy-confirmed NASH[1].

Method: Pts with NASH and fibrosis stages F2-3 (ITT), and an exploratory group of F1 pts with metabolic syndrome, were randomized to placebo (PBO), OCA 10mg, or OCA 25mg QD. Primary end points were fibrosis improvement (≥1 stage) with no worsening of NASH, or NASH resolution with no worsening of liver fibrosis per liver biopsy. The safety population included all randomized and dosed pts (F1-3, N = 1968). Clinical outcomes will be evaluated at the end-of-study.

Results: The ITT population included 931 pts (PBO [n = 311], OCA 10mg [n = 312] or OCA 25mg [n = 308]), comprised of 44% F2 and 56% F3. Baseline characteristics were well-balanced across groups. Results in Table. The primary fibrosis end point was met by 11.9% PBO, 17.6% OCA 10mg (p = 0.0446 vs PBO), and 23.1% OCA 25mg (p = 0.0002 vs PBO) pts (ITT). The primary NASH end point was not statistically significant (ITT); however significantly more pts on OCA 25mg showed improvements in hepatocellular ballooning (p = 0.0011 vs PBO) and lobular inflammation (p = 0.0322 vs PBO). Dose-dependent reductions in ALT, AST and GGT were observed. Pruritus was the most common AE (19% PBO, 28% OCA 10mg, 51% OCA 25mg) and was predominantly mild to moderate in severity (severe pruritus: <1% PBO, <1% OCA 10mg, 5% OCA 25mg). SAEs occurred in 11% PBO, 11% OCA 10mg and 14% OCA 25mg pts. Increases in LDLc with OCA were observed by Week 4, but approached baseline by Month 18 (OCA 25mg: LS mean change Wk4 +22.6 mg/dL, M18 +4.0 mg/dL). Three deaths occurred; none were considered treatment-related (PBO n = 2; OCA 25mg n = 1).

Conclusion: Treatment with OCA 25mg improved liver fibrosis, key histologic features of steatohepatitis and liver biochemistry, demonstrating consistent efficacy with an overall AE profile similar to previous studies.

[1]Interim analysis results at 18 months are based on surrogate end points and impact on clinical outcomes has not been confirmed. The REGENERATE study is ongoing to confirm the clinical benefit of OCA.

Figure:

	Placebo	OCA 10 mg	OCA 25 mg
Primary: ITT Population (F2 + F3)	**n = 311**	**n = 312**	**n = 308**
Fibrosis improvement + no worsening of NASH	11.9%	17.6% p = 0.0446	23.1% p = 0.0002
NASH resolution + no worsening of fibrosis	8.0%	11.2% p = 0.1814	11.7% p = 0.1268
Improvement in hepatocellular ballooning	23.2%	27.2% p = 0.2423	35.1% p = 0.0011
Improvement in lobular inflammation	35.7%	39.1% p = 0.3380	44.2% p = 0.0322

Overall study discontinuations (ITT): 16% PBO, 17% OCA 10 mg, 15% OCA 25 mg.

P05-22YI Burden of non-alcoholic fatty liver disease in helicobacter pylori infected dyspeptic patients, is it a far reaching implication?

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Background and aims: Fatty infiltration of the liver represents intracytoplasmatic accumulation of triglycerides in the hepatocytes. We determined if *H. pylori* infection contributes to fatty liver infiltration and if it was related to gastric mucosal changes and fatty liver disease.

Method: In this cross-sectional study, patients underwent investigations for *H. pylori* infection. Body mass index (BMI) was calculated. Normal BMI was upto 22.9 and abnormal 23 and greater. Data was collected for age, gender, smoking, alcohol intake, hypertension, type II diabetes, ischemic heart disease, dyslipidemia and liver function tests. Ultrasound of liver diagnosed fatty liver infiltration.

Results: 698 patients were enrolled with mean age 44 ± 16. Male were 373 (53%). *H. pylori* infection was positive in 399 (57%). Fatty liver was documented in 153 (22%). In *H. pylori* infection, fatty liver was positive in 31-50 year 31 (35%) and in 51-65 year 37 (42%) and was absent in 127 (41%) and 88 (28%) (p <0.001) in these groups, respectively. In patients with *H. pylori* infection, BMI greater than 23, liver fatty infiltration was present in 84 (26%) (p <0.001) compared to 4 (5%) in BMI less than 23. *H. pylori* infection induced chronic active gastritis was associated with fatty liver infiltration in 62 (71%) and absent in 200 (64%) (p = 0.264).

Conclusion: *H. pylori* infection was associated with an early onset of fatty liver infiltration in the 30-50 year age group. Patients with a BMI >than 23, type 2 diabetes and dyslipidaemia with *H. pylori* infection predisposed to fatty liver. The complex interaction of gut microbiota and H.pylori infection promotes NAFLD through GUT-LIVER-AXIS.

Figure:

Table. Clinical details with liver fatty infiltration and *H. pylori* Status							
		Helicobacter pylori positive (n = 399)			Helicobacter pylori negative (n = 299)		
		Fatty infiltration liver			Fatty infiltration liver		
		Positive	Negative	P value	Positive	Negative	P value
Age (year)	18-30	5 (6)	73 (23)	<0.001	3 (5)	78 (33)	<0.001
	31-50	31 (35)	127 (41)		15 (23)	51 (22)	
	51-65	37 (42)	88 (28)		40 (61)	79 (34)	
	65 and >	15 (17)	23 (7)		7 (11)	26 (11)	
Sex	Male	41 (47)	168 (54)	0.218	35 (54)	129 (45)	0.854
	Female	47 (53)	143 (46)		30 (46)	105 (76)	
BMI	Upto 23	4 (4)	76 (24)	<0.001	10 (15)	114 (49)	<0.001
	23 and >	84 (96)	235 (76)		55 (85)	120 (51)	
Hypertension	Positive	36 (41)	27 (9)	<0.001	15 (23)	11 (5)	<0.001
	Negative	52 (59)	284 (91)		50 (77)	223 (95)	
Type 2 Diabetes	Positive	46 (52)	27 (9)	<0.001	15 (23)	12 (5)	<0.001
	Negative	42 (48)	284 (91)		50 (77)	222 (95)	
Dyslipidemia	Positive	47 (53)	26 (8)	<0.001	16 (25)	16 (7)	<0.001
	Negative	41 (47)	285 (92)		49 (75)	218 (93)	
Ischemic Heart Disease	Positive	33 (37)	19 (6)	<0.001	16 (25)	13 (6)	<0.001
	Negative	55 (63)	292 (94)		49 (75)	221 (94)	

Histology							
Gastritis	Mild	39 (44)	181 (58)	0.021	21 (32)	127 (54)	0.002
	Moderate	49 (56)	130 (42)		44 (68)	107 (46)	
Grade	Chronicactive gastritis	62 (71)	200 (64)	0.264	55 (85)	171 (73)	0.055
	Chronic inflammation	26 (29)	111 (36)		10 (15)	63 (27)	

P06-01YI miR-34a is strongly activated in human and experimental NAFLD, correlating with key disease hallmarks

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) pathogenesis remains incompletely understood. In this regard, we and others have shown that microRNAs (miRNA/miRs), particularly miR-34a, are key modulators of disease progression, from steatosis to non-alcoholic steatohepatitis (NASH), up to development of hepatocellular carcinoma. We now aimed to elucidate whether activation of miR-34a represents a universal event in etiological diverse diet-induced NAFLD models and which disease hallmarks correlate with miR-34a expression, pinpointing its main roles in disease pathogenesis.

Method: C57BL6 mice were fed five different NAFLD-inducing diets, namely a methionine and choline-deficient diet for 2 and 8 weeks; a high-fat choline-deficient diet for 14 weeks; a high fat, 2% cholesterol diet for 25 weeks; a high-fat/calorie diet with high fructose/glucose in drinking water for 16 weeks; and a choline-deficient amino acid-defined diet for 32 weeks. Liver biopsies were obtained from a cohort of 165 NAFLD patients, thoroughly characterized at the histological and metabolic levels (NAS≤2: n = 26; NAS 3 to 4: n = 62; NAS≥4: n = 77). miRNAs were quantified by Taqman Advanced Real-Time RT-PCR.

Results: Mice fed any of the five diets developed different degrees of steatosis, NASH and fibrosis, with or without significant weight gains or development of insulin resistance. Nonetheless, liver miR-34a expression levels were significantly increased in all diseased mice ($p < 0.05$), comparing with control diet-fed animals.

NAFLD patients exhibited different degrees of steatosis and NASH, with or without the presence of fibrosis and concomitant diseases. Liver miR-34a expression was found to progressively increase with steatosis, lobular inflammation and NAS score ($p < 0.05$ for all). Furthermore, miR-34a expression levels were significantly increased in patients with advanced fibrosis ($p < 0.05$), as well as in those with concomitant diabetes, arterial hypertension and cholelithiasis ($p < 0.05$). miR-34a expression also progressively increased with age, although women displayed lower expression levels comparing to men ($p < 0.01$). Finally, bivariate analysis indicated that liver miR-34a expression positively correlated with histological findings (steatosis, lobular inflammation, fibrosis and NAS score), serum hepatic enzymes (AST and ALT), hepatic triglyceride content and age ($p < 0.05$).

Conclusion: In conclusion, activation of miR-34a appears to be a key event governing NAFLD pathogenesis and progression, correlating with specific, well-characterized disease hallmarks. (Gilead Sciences International-Research Scholars Program in Liver Diseases; PTDC/MED-PAT/31882/2017 and SFRH/BD/104160/2014, FCT, Portugal).

P06-03 Serum adenosine deaminase values are associated with advanced liver disease in patients with Non-Alcoholic Fatty Liver Disease

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Background and aims: Adenosine deaminase (ADA) and its isoenzyme Adenosine deaminase 2 (ADA2), are enzymes of the purine metabolism with important effects on the regulation of the immune system. Recent data suggested that the expression of these enzymes in portal macrophages is also associated with inflammation and liver fibrosis in patients with Non-Alcoholic Fatty Liver Disease (NAFLD). The aim of this study was to assess the diagnostic value of serum ADA and ADA2 in predicting liver fibrosis stages in patients with NAFLD and compare its accuracy to conventional indices of liver fibrosis.

Method: We prospectively included patients with biopsy proven NAFLD. Fibrosis-4 (FIB-4), NAFLD Fibrosis Score (NFS), and AST to Platelet Ratio Index (APRI) were calculated using the corresponding formulas. Total ADA and ADA2 activity in the serum was evaluated using standard enzymic assays. To evaluate the diagnostic value of ADA and ADA2, receiver operating curve (ROC) analysis was performed. Statistical analysis was performed using the SPSS software.

Results: We enrolled 26 patients (males, 50%) with median (range) age of 56 (37- 77) years. The median BMI was 28, 4 (22, 1-50). The distribution of liver fibrosis stages was: F1 = 4 (15, 4%), F2 = 9 (34, 6%), F3 = 5 (19, 2%), and F4 = 8 (30, 8%). Laboratory values were as follows (median [range]): PLT 212.500 (35.000-425.000); AST 33 (15-120) IU/ML; ALT 36 (14-108) IU/ML; γ-GT 64 (10-349) IU/ML; ALP 84 (44-126) IU/ML; NFS 1.551 (-5.299-5.272); FIB-4 1.71 (0.46-17.84); APRI 0.4435 (0.168-1.422); ADA 7.9 (2.6-37.6); ADA2 7.3 (2-33.7). The performance of ADA and ADA2 in predicting cirrhosis was superior to other indices with an area under the ROC (AUROC) of 0.975 and 0.992, respectively (AUROC for NFS, FIB-4 and APRI were: 0.916; 0, 933; 0, 731). In addition, total ADA had similar diagnostic value with FIB-4 in predicting severe fibrosis (F3-F4) (AUROC of 0.955 vs. 0.958), whereas ADA2 was inferior to NFS and APRI (AUROC of 0.927 vs. 0.937 and 0.868, respectively) in the same population. Finally, both ADA and ADA2 were inferior to all other indices in predicting significant fibrosis (F2-F4) with an AUROC of 0.644 and 0.588, respectively (AUROC for NFS, FIB-4 and APRI were: 0.813; 0.863; 0, 763).

Conclusion: Serum ADA and ADA2 seem to be accurate and easy to use biomarkers for the diagnosis of advanced liver disease (especially cirrhosis) in patients with NAFLD. Large-scale studies are warranted to confirm these promising preliminary results.

P06-04 Diagnostic performance of three non-invasive fibrosis scores (Hepamet, Fib-4, Nafld Score) on NAFLD in a mixed Brazilian population

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Background and aims: A variety of non-invasive fibrosis scores have been to estimate risk of fibrosis in Non-alcoholic fatty liver disease (NAFLD) have been developed in different cohorts in the world. Our aim was to evaluate in the mixed Brazilian population the performance of three differents non-invasive scores in NAFLD patients.

Method: Biopsy-proven NAFLD patients from a single tertiary center were included in the study. HEPAMET, the new Latin NAFLD score, FIB4 and NAFLD score were determined in the mixed Brazilian population. Fibrosis was staged according Kleiner score. Significative fibrosis was considered if patients had F2, F3, F4, advanced fibrosis F3-F4, and cirrhosis (F4). Diagnostic accuracy was assessed according to AUROC sensitivity, specificity values for the diagnosis of sifnificative, advanced fibrosis, cirrhosis and were calculated to evaluate the performance of these non-invasive methods in NAFLD patients, adopting liver biopsy as the gold standard and correct classification and grey zone were done. The comparison of AUROC curves was done by Hanley test.

Results: A total of 179 biopsy-proven NAFLD patients were analyzed. About seventy six of them (76.53%) were female, median age 56 (48-62) years, body mass index BMI 32 (28-35.1) and 84.91% had glucose intolerance or diabetes. Liver fibrosis were showed F1 (60.89%), F2 (15.64%), F3 (16.75%), F4 (6.70%) in this population. The Hepamet, FIB 4 and NAFLD Fibrosis Score models had a high accuracy for the diagmosis of significative and advanced fibrosi and the presence of cirrhosis. FIB-4, Hepamet and NAFLD score were similar and there is no difference between the scores (AUROC 0.756; AUROC 0.7616; AUROC 0.6895 p > 0.05) (Table 1) even in a mixed population.

Conclusion: The use of Hepamet Score, FIB 4 and NFS models in Brazilian NAFLD population allows a diagnosis severe liver disease to be excluded. The three scores can be use in NAFLD patients as na first tool to predict significative, advanced fibrosis and cirrhosis even in a mixed population.

Figure: 1

Significative Fibrosis

NFS			HEPAMET			FIB4	
Sensitivity:	83%		Sensitivity:	65,70%		Sensitivity:	53,60%
Specificity:	34%		Specificity:	60,60%		Specificity:	81,40%
Accuracy:	36,90%		Accuracy:	62,60%		Accuracy:	70,20%
AUROC	0,7007		AUROC	0,6836		AUROC	0,691
Std. Dev. (Area)	0,0985		Std. Dev. (Area)	0,0543		Std. Dev. (Area)	
			P (Hanley test)	0,76			

Advanced fibrosis

NFS			HEPAMET			FIB4	
Sensitivity:	78,57%		Sensitivity:	76,20%		Sensitivity:	76,90%
Specificity:	35,77%		Specificity:	58,40%		Specificity:	63,40%
Accuracy:	45,80%		Accuracy:	62,60%		Accuracy:	73,70%
AUROC	0,6895		AUROC	0,7616		AUROC	0,735
Std. Dev. (Area)	0,061		Std. Dev. (Area)	0,0542		Std. Dev. (Area)	
			P (Hanley test)	0,28			

Cirrhosis

NFS			HEPAMET			FIB4	
Sensitivity:	83,30%		Sensitivity:	91,70%		Sensitivity:	75%
Specificity:	33,50%		Specificity:	53,30%		Specificity:	70,40%
Accuracy:	36,90%		Accuracy:	55,90%		Accuracy:	70,80%
AUROC	0,7007		AUROC	0,8219		AUROC	0,8041
Std. Dev. (Area)	0,0985		Std. Dev. (Area)	0,0592		Std. Dev. (Area)	0,0768
			P (Hanley test)	0,29			

P06-05 Establishment of a 3D human liver model to recapitulate NASH progression in vitro

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is the most prevalent type of liver disease and currently affects ~30% of the population. With progression to non-alcoholic steatohepatitis (NASH), this disease can eventually lead to liver cirrhosis and failure. To date, there are no approved drugs for NASH treatment and drug development has been impeded by the lack of predictive *in vitro* models reflecting the complex pathology of NASH. Our human in vitro 3D NASH model is engineered to incorporate the primary human hepatocytes, hepatic stellate cells, Kupffer cells and liver endothelial cells, all playing a crucial role in disease progression.

Method: We developed a protocol for induction of NASH with free fatty acids and LPS in medium containing high levels of sugars to recapitulate NASH pathogenesis and for drug efficacy testing *in vitro* and analysed characteristic markers like lipid loading using triglyceride assays, secretion of proinflammatory markers (Luminex) and fibrosis (pro-collagen type I secretion).

Results: Upon treatment with free fatty acids and LPS in diabetic medium containing high levels of sugars these microtissues showed key physiological aspects of NASH. Increased lipid accumulation within the hepatocytes and tissue triglyceride levels were detected in the NASH treated tissues as compared to the control treated tissues., NASH stimuli increased the secretion of pro-inflammatory markers, such as TNF-α, IL-6, IL-8, MCP-1, MIP-1α and IP-10. Furthermore, NASH stimuli increased the secretion of pro-collagen type I and the deposition of fibril collagens type I/III, whereas the TGFRβ1 inhibitor (ALK5 inhibitor) dose-dependently decreased the expression of those pro-fibrotic markers.

Conclusion: In summary, we present a human 3D NASH model that recapitulates key biological aspects such as inflammation, steatosis and fibrosis. Compatible with high-throughput screening approaches, this model is a powerful tool for assessing efficacy of anti-NASH drugs.

P06-06YI Evaluating accuracy of serum steatosis and fibrosis scores in a cohort of morbid obese patients

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Background and aims: Non-alcoholic fatty liver desease (NAFLD) is actually the leading cause of end-stage liver desease. In obese subjects is common but underdiagnosed. Liver biopsy is the gold standard to identify patients with non-alcoholic steatohepatitis and early fibrosis. However, its invasive nature, make it less suitable in clinical practice. The aim of this study is to determine the diagnostic accuracy of common non-invasive diagnostic tests for steatosis and fibrosis in a cohort of morbid obese patients undergoing bariatric surgery.

Method: We recruited 92 obese subjects (age 40.4 ± 11.5 years, body mass index 44.8 ± 14.1 kg/m²) undergoing bariatric surgery. Medical history, blood tests and oral glucose tolerance test (OGTT) were taken as preparation for surgery. Intraoperative liver biopsy was taken on the day of surgery in all patients. Serum steatosis and fibrosis scores (NAFLD-LFS, FLI, NFS, FIB4) were calculated with standardized formulas and liver ultrasound with acoustic radiation force impulse (ARFI) was performed.

Results: Histologically, the prevalence of NAFL was 61.95% (n = 57), NASH 23.91% (n = 22). The prevalence of fibrosis was: F0 8.9%, F1 76.7%, F2 13.3%. Advanced fibrosis (F3) was found in one patient only (1.1%) and nobody was F4. Serum steatosis scores were calculated: NAFLD-LFS score was 2, 3 ± 2, 3 in NAFL group patients and 2, 1 ± 2, 1 in NASH group. FLI was 85, 5 ± 28, 6 in NAFL group and 61, 2 ± 46, 4 in NASH group. Both resulted above cut off value (60 for FLI, -0.64 for NAFLD LFS) in control group patients (81 ± 32, 9 and 2, 2 ± 2, 2 for FLI and NAFLD LFS respectively) indicating a lack of accuracy in morbid obese patients, probably due to the high BMI values for FLI formula. Serum fibrosis score were calculated: FIB4 was 0, 7 ± 0, 5, 0, 6 ± 0, 2 and 0, 66 ± 0, 3 in control group, NAFL and NASH group respectively, resulting below cut off for advancing fibrosis that is 6.45. NFS was -0, 8 ± 0, 5, -0, 9 ± 1, 2 and 1, 9 ± 3, 9 in control group, NAFL and NASH group respectively. NFS resulted diagnostic for advanced fibrosis in NASH group. ARFI was 7, 1 ± 10, 7, 3 ± 11, 3, 2 ± 1, 7 in control, NAFL and NASH group patients respectively, resulting diagnostic for advanced livers fibrosis in all group of patients.

Conclusion: In patients with morbid obesity: FLI and NAFLD-LFS are not accurated in morbid severe patients because they overestimate the degree of steatosis. FIB4 is suitable because it excludes the presence of advanced fibrosis. NFS and ARFI appear innacurate.

P06-07YI Circulating mir-192-5p as a novel biomarker for liver fibrosis progression to cirrhosis

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Background and aims: MicroRNAs are attractive biomarker candidates for diagnostic purposes as microRNA expression within tissues dramatically changes in disease states. Moreover, in response to injury, cell-type specific microRNAs can be released into circulation, allowing for non-invasive biopsies to diagnose disease as well as stratify patients based on disease severity.

Cirrhosis is currently the 11th most common cause of death globally and hepatocellular carcinoma (HCC) is the 16th leading cause of death. The most prevalent liver disease is non-alcoholic fatty liver disease (NAFLD). Despite this, there remains a paucity of early diagnostic biomarkers for liver disease.

This study aimed to:

Identify circulating microRNAs that are differentially expressed in patients with increasing fibrosis grading (Discovery phase).

Validate selected microRNAs in a larger cohort (Validation phase).

Evaluate the functional role of selected microRNAs by *in vitro* assays.

Method: For the Discovery phase, microRNAs present in RNA from patients with a fibrosis score of F0 (n = 5), F1-2 (10), and F4 (n = 16) were analysed on the *Nanostring nCounter* platform (798 microRNA targets). Data were input into the manufacturer's software nSolver for QC and normalisation to the top 100 microRNAs detected in all groups.

For the Validation phase, RNA from Healthy (n = 25), NAFLD/NASH (n = 20), Cirrhosis (n = 33), and HCC (n = 28) patient plasma samples was profiled for mir-192-5p expression using the -ddCt method.

Results: Of 798 microRNAs examined, 5 microRNAs (mir-192-5p, mir-612, mir-323-3p, mir-526a, and mir-376a-3p) showed increasing expression with fibrosis grade, with mir-192-5p showing the strongest trend. Mir-192-5p was then selected for further validation as a circulating biomarker of liver fibrosis.

Circulating mir-192-5p is significantly elevated in cirrhosis patients regardless of disease aetiology and can distinguish between healthy and cirrhosis (p = 0.0002). Circulating mir-192-5p is also significantly elevated in NAFLD/NASH and can distinguish between healthy and NAFLD/NASH (p = 0.0008).

Circulating levels of mir-192-5p is significantly elevated in NASH compared to Healthy and NASH HCC and can distinguish between Healthy and NASH (p = 0.002), NAFLD and NASH (p = 0.0025), and NASH and NASH HCC (p = 0.0018).

Mir-192-5p is significantly downregulated in HCC tumours compared to adjacent liver tissue and is non-significantly elevated in colorectal metastases compared to adjacent liver tissue.

Conclusion: These data show that mir-192-5p is a promising circulating biomarker of liver fibrosis progression to cirrhosis. We observed the greatest trend of increasing expression in our NAFLD/NASH/NASH HCC cohort, where mir-192-5p expression increases from Healthy to NAFLD to NASH and decreases in NASH HCC.

P06-08 Non-electrophilic activation of the NRF2 pathway ameliorated experimental Non-alcoholic Steatohepatitis

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Background and aims: Oxidative stress and inflammation play a key role in a wide range of diseases. The transcription factor Nrf2, mediates adaptation to oxidative stress by inducing antioxidant and cytoprotecting genes. Recent studies show Nrf2 activation has multiple benefits which include the decrease oxidative stress, enhanced redox capacity, anti-inflammation, modulation of lipogenesis and gluconeogenesis, regulation of autophagy, proteostasis and mitochondrial biogenesis and energetics. Pharmacological activation of the Nrf2 pathway has been recognized as a potential strategy to reduce oxidative stress and resolve inflammation associated with acute and chronic illnesses.

vTv Therapeutics has developed non-electrophilic, orally bioavailable molecules which activate the Nrf2 pathway by inhibition of Bach1 transcriptional repression and stabilization of Nrf2. Numerous in vitro and in vivo studies have demonstrated that modulation of NRF2 by these compounds results in reduction of both oxidative stress and inflammation. However, unlike electrophiles, these drugs are not reactive, their effect is not suppressed by N-acetyl cysteine, and they do not perturb either ROS or cellular glutathione. Non-alcoholic steatohepatitis (NASH) is a progressive liver disease highly associated with oxidative stress, inflammation, lipid metabolism. The aim of the present study was to evaluate the therapeutic potential of the non-electrophilic Nrf2 activator HPP-3033 in a MCD diet-induced NASH model in mice

Method: Mice were fed a choline methionine deficient diet for 4 weeks. After 1 week of diet, mice were randomized into 3 treatment groups according to 1) ALT, 2) AST and 3) body weight. After randomization, mice were treated QD for 3 weeks with vehicle (n = 10), HPP3033 (n = 10) and FXR agonist WAY-362450 (n = 10). At the end of the treatment period blood and liver samples were collected for histology and biomarker quantification. Data are shown as mean ± SEM. Statistical analysis was performed using a 1-way or 2-way ANOVA

Results: HPP-3033 treated mice displayed significant decrease in hepatocyte ballooning ($0.6 ± 0.22$, vs $0 ± 0$ $p < 0.05$, for vehicle and HPP-3033 respectively), Moreover, treatment with HPP-3033 displayed trends towards lowering liver lipids and inflammation in the liver, leading to a significant reduction in total NAS score ($5.6 ± 0.28$ and $4.0 ± 0.21$ $p < 0.01$ for vehicle and HPP-3033, respectively). Target engagement was confirmed by a significant increase in HMOX1, a gene which expression is highly controlled by Bach1 repression (8-fold $p < 0.01$ vs. vehicle).

Conclusion: In the methionine choline deficient diet fed mouse model, treatment with 3033 resulted in higher gene expression of heme oxygenase and significant improvement of NAS score, confirming the potential of vTv's non-electrophilic NRF2 activators to be developed as a treatment option for NASH.

P06-09 Dietary switch and exercise differentially resolove NASH and fibrosis

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Background and aims: Non-alcoholic steatohepatitis (NASH) has become an increasingly common chronic liver disease in developed countries, paralleling the increased prevalence of obesity. Lifestyle recommendations remain the primary treatment for NASH patients. Due to absence of major randomized controlled trials of lifestyle changes for NASH, the specific effects of dietary changes and exercise on NASH and underlying molecular pathways involved, remain elusive. We therefore investigated the effects of dietary change, exercise, and the combination thereof on already manifest NASH/fibrosis in mice.

Method: Ldlr-/-.Leiden mice received a high fat diet for 30 weeks to induce NASH/fibrosis. After 30 weeks mice were left untreated (control group) or switched to an isocaloric healthy chow diet, received a running wheel, or the combination thereof, for another 20 weeks. Effects on body weight, plasma and liver biochemical variables, liver histology, adipose tissue (inflammation and adipocyte size) were assessed.

Results: Both dietary change and exercise significantly reduced body weight, fat mass, adipocyte size and plasma ALT levels and the combination treatment had additive effects. While exercise significantly reduced micro-vesicular steatosis but had no effects on macro-vesicular steatosis, dietary change reduced both forms of steatosis with significant effect on macro-vesicular steatosis. The combination treatment revealed additive effects for both macro- and micro-vesicular steatosis.

Hepatic inflammation was almost fully reduced by each of the monotreatment. Adipose tissue inflammation was significantly reduced by dietary switch, tended to be reduced by exercise, without additive effects in the combination group. Fibrosis was significantly reduced by dietary switch, tended to be reduced by exercise, and combined treatment had no additive resolving effect.

Conclusion: Both dietary and exercise lifestyle interventions showed beneficial effects in mice with clear additive effects for the combination treatment for aspects related to lipid handling (steatosis and adipocyte size) and beneficial effects without additive effects for hepatic inflammation and fibrosis. This suggests the metabolic effects of the interventions related to lipid handling are mechanistically complementary and therefore additive, while the inflammatory and fibrotic reductions may involve similar pathways that do not add up.

P06-10YI Oxidized-LDL as a marker of oxidative stress is strongly related to NASH irrespectively of insulin resistance and liver fibrosis

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Background and aims: Insulin resistance and oxidative stress are main drivers for NAFLD, but their relationship has been not completely assessed in clinical practice. We aimed to determine the role of oxidative stress on NAFLD severity and elucidate whether it shows a different clinical phenotype than insulin resistance.

Method: This was a prospective-designed, cross-sectional, multicenter study including 270 patients that underwent liver biopsy due to clinical suspicion of NAFLD. Epidemiological, biochemical and anthropometrical variables were recorded. Oxidative stress was measured by oxidized LDL levels (ELISA, Mercodia AB, Uppsala, Sweden), while insulin resistance was defined by HOMA $\geq$4. NASH was evaluated by using NAS Score and liver fibrosis by Kleiner score.

Results: Two hundred and seventy patients were included: NASH (n = 72); NAFL (n = 133) and patients with normal liver histology (n = 65). Fibrosis distribution was: F0 39.3% (106/270), F1 31.5% (85/270), F2 18.9% (51/270), F3 7.4% (20/270) and F4 3% (8/270). OxLDL levels in healthy controls were 47.1$\pm$12.5 U/L, while it was 52.7$\pm$15.7 U/L in NAFL and 56.1$\pm$16.1 U/L in NASH (p < 0.0001). There was no link with liver fibrosis (F0 53.4$\pm$15.2 *vs.* F1 52.1$\pm$16.3 *vs.* F2 49.8$\pm$14 *vs.* F3 57.7$\pm$16.1 *vs.* F4 43.6$\pm$9.2 U/L; p = 0.134). Age, male sex, diabetes mellitus (DM), arterial hypertension, BMI, AST, ALT, HOMA, and triglycerides were also associated to NASH. After multivariate analysis, oxLDL [OR 1.03 (CI95% 1.01-1.05); p = 0.010], BMI [OR 1.07 (CI95% 1.04-1.11); p = 0.0001], age [OR 1.04 (CI95% 1.01-1.07); p = 0.004] and AST [OR 1.01 (CI95% 1.00-1.03); p = 0.040] remained as independent variables related to NASH. Further, we categorized patients according to insulin resistance (HOMA$\geq$4) or DM *versus* oxidative stress (oxLDL$\geq$51, cut-off selected after ROC analysis). Patients with only oxidative stress showed significantly lower BMI, AST, ALT and fibrosis stage than those with insulin resistance. Also, subjects with insulin resistance showed greater impairment in carbohydrate metabolism, while lipid profile was more altered in patients with oxidative stress (Table).

Conclusion: oxLDL levels were associated to NASH, but did not to liver fibrosis. The clinical phenotype of patients with oxidative stress was different from those with insulin resistance. These data may reinforce the role of oxidative stress in NASH, independently of obesity and diabetes, highlighting the role of this molecule linking NASH and cardiovascular risk.

Figure:

Variable	None	Oxidative Stress	Insulin Resistance	Both	P value
Age	45.2±11.7	49.1±12.1	52.4±12.5	48.1±12.6	0.014
IMC	36.2±11	34.7±11.7	41.8±8.8	39.9±9	0.001
DM	0%	0%	69.4%	62.3%	0.0001
Arterial Hypertension	27%	34.5%	69.4%	54.7%	0.0001
AST	29±22	24±17	33±25	38±35	0.042
ALT	39±35	29±19	38±25	57±54	0.0001
Glucose	92±13	95±13	125±34	120±40	0.0001
Insulin	8.1±4.2	8.4±4.4	19.5±13.8	20±10.1	0.0001
HOMA	1.8±0.9	2±1	5.8±4.3	5.9±3.8	0.0001
Hb1Ac	5.1±0.5	5.2±0.5	6.6±1.6	6.4±1.6	0.001
Total cholesterol	183±33	212±44	166±32	200±33	0.0001
HDL-c	51±12	52±22	41±10	44±11	0.0001
LDL-c	113±24	142±32	96±30	122±30	0.0001
Triglycerides	113±61	126±53	158±76	167±77	0.0001
OxLDL	40.8±7.4	64.4±12.8	40.3±7.6	64.6±9.8	0.0001
NAS score	2.49±2.01	2.79±2.25	3.31±1.67	3.60±1.89	0.010
Fibrosis stage	0.77±85	0.66±0.97	1.49±1.23	1.25±1.09	0.0001

P06-11 Elafibranor, a drug candidate for first line NASH monotherapy and a universal backbone for drug combination treatment

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Background and aims: Elafibranor (ELA), a PPARα/δ agonist, reverses NASH histology and decreases fibrosis in patients with advanced disease [1], and is currently being evaluated in the RESOLVE-IT phase 3 trial. Among other NASH treatment candidates, obeticholic acid (OCA), GS-0976 (GS), selonsertib (SEL) and cenicriviroc (CVC) have complementary actions with ELA.
Aim: to provide experimental evidence for ELA as a universal backbone for drug combination therapies in NASH

Method: C57Bl/6J mice and Wistar rats were fed a fat and/or cholesterol supplemented choline deficient CDAA diet (modified CDAA) for 8-12 weeks to generate NASH with fibrosis. Histological evaluation was performed in a blinded fashion. NASH was assessed using the NASH Clinical Research Network Scoring System. Fibrosis area was determined by measuring the collagen positive area.

Results: The animals developed a severe NASH phenotype with abundant fibrosis.
Low doses of ELA (1 and 3 mg/kg) decreased NAFLD activity score (NAS) by 1 ($p < 0.0001$) and 2 points ($p < 0.0001$), respectively. Among the other compounds, only GS (10 mg/kg) reduced NAS by 1 point ($p = 0.04$) under conditions used in this study. Combination of ELA with GS (10 mg/kg) or CVC (10 mg/kg) but not with other compounds, improved NAS by 3 points ($p < 0.001$ for both compounds).
Monotherapy with ELA (1 mg/kg), ELA (3 mg/kg), OCA (10 mg/kg), SEL (30 mg/kg) and GS (10 mg/kg) reduced fibrosis area by 24% ($p = 0.0006$), 47% ($p < 0.0001$), 26% ($p = 0.15$), 32% ($p = 0.02$) and 38% ($p = 0.0001$), respectively. Further, OCA and SEL in combination with ELA decreased fibrosis area by 71% ($p < 0.0001$) and 49% ($p < 0.0001$), respectively. The effect of GS on fibrosis was not further potentiated by ELA.
PK studies in rodents did not show any relevant drug-drug interactions with respect to plasma exposure following ELA coadministration with OCA, SEL and CVC. The interaction with GS cannot be excluded and is currently under investigation.

Conclusion: ELA worked in synergy with most of the other candidates to efficiently attenuate fibrosis development. Use of drug combinations in this study preserved treatment efficacy at reduced therapeutic doses. We conclude that ELA is a universal backbone for combination drug therapies in NASH.

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P06-12 A high-cholesterol diet promotes steatohepatitis and liver tumorigenesis in HCV core gene transgenic mice

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Background and aims: Previous epidemiological studies have suggested a link between high cholesterol intake and liver disease progression, including hepatocellular carcinoma (HCC). However, the precise mechanism of hepatotoxicity and hepatocarcinogenesis caused by excessive cholesterol consumption remains unclear. We aimed to investigate the impact of dietary cholesterol using hepatitis C virus core gene transgenic mice (HCVcpTg), which spontaneously developed HCC with age.
Method: Male HCVcpTg mice were treated for 15 months with either a purified control diet or an isocaloric diet containing 1.5% cholesterol, and liver phenotypes and tumor-associated signaling pathways were evaluated.
Results: The high-cholesterol diet-fed HCVcpTg mice exhibited a significantly higher incidence of liver tumors compared with the control diet mice (100% vs. 41%, $p < 0.001$). The diet induced steatohepatitis with pericellular fibrosis and evoked higher mRNA expression of pro-inflammatory and pro-fibrotic mediators along with enhanced hepatocyte proliferation and greater oxidative and endoplasmic reticulum stress in the liver. Moreover, long-term consumption of cholesterol-rich diet activated nuclear factor-kappa B (NF-κB) and p62/sequestosome 1 (Sqstm1)-nuclear factor erythroid 2 (NRF2) axis, enhanced fibrogenesis, and consequently accelerated hepatic tumorigenesis.
Conclusion: These results demonstrate that a high-cholesterol diet facilitates liver tumorigenesis by inducing steatohepatitis, promoting hepatocyte division, and up-regulating cellular stress and pro-inflammatory NF-κB and detoxifying p62/Sqstm1-NRF2 signals. Therefore, high dietary cholesterol should be avoided in HCV-infected patients to prevent development of steatohepatitis, liver fibrosis, and HCC.

P06-13YI Changes in autophagic flux in diet-induced non-alcoholic fatty liver disease and hepatic fibrosis models in C57Bl/6 mice

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Background and aims: Autophagy is the biological process of degradation of aberrant proteins and damaged organelles for cell survival and seen as an alternative pathway to apoptosis in cellular stress. Normally activated during starvation states, impaired autophagic flux due to lipotoxicity in hepatocytes may contribute to liver injury and fibrogenesis. Macroautophagy in lipid metabolism, lipophagy, has been observed to increase with high-fat diets but decrease with cholesterolaemic saturated fatty acids such as palmitic acid. Changes in autophagic flux with progressive as well as resolution of steatotic injury disease is poorly characterised.

Our study aims to identify changes in autophagy with disease progression and resolution from injury in; 1) high fat diet (HFD) murine model, 2) HFD and cholesterol diet and 3) a non steatotic injury thioacetamide-induced (TAA) model.

Method: For diet-induced fatty liver induction, 8-week old male C57Bl/6 mice were given HFD (45% kcal fat) or an atherogenic diet (HFD+C) (45% kcal fat + 0.25% cholesterol) for 6, 24 weeks followed by 6 weeks of normal chow (resolution). TAA was administered in drinking water (300mg/L *ad libitum*) at equivalent times. Control were given a normal chow diet (12%kcal fat). Whole liver was taken for mRNA and immunoblotting for LC3B as a marker of macroautophagy.

Results: Mice in HFD or HFD+C groups gained body mass as similar rates over 24 weeks of feeding, significantly different from control groups from week 11 onwards ($p < 0.05$), and similarly returned to chow weights at withdrawal of feed. TAA-treated animals were lighter than control animals, which then equilibrated with resolution from injury. All cohort exhibited increasing serum ALP, ALT and AST with increasing liver injury and fibrosis.

Intrahepatic LC3B mRNA expression in HFD and HFD+C increased at 6 weeks of feeding and then decreased in both feed groups compared to normal chow fed mice once liver injury and steatosis was established (24 weeks). Similarly, with the HFD and HFD+C LC3B-II protein expression increased at 6 weeks and remained elevated to 24 weeks. LC3B-II levels equilibrated after recovery for 6 weeks. At all timepoints, LC3B-I protein expression did significantly change compared to controls. In TAA-treated mice, LC3B gene and protein expression (LC3B-I and LC3B-II) was decreased with injury at all timepoints and equilibrated to uninjured level with recovery.

Conclusion: Changes in autophagic flux was observed in the livers of mice fed a high fat or atherogenic diet. Further differences in macroautophagy appear to be injury dependant and differ comparing progressive and established injury. Therefore, the time course of autophagy changes required further investigation in the development of steatotic liver injury.

P06-14 The smoking-hemoglobin interaction and risk for advanced fibrosis in patients with biopsy-proven non-alcoholic fatty liver disease

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Background and aims: Higher hemoglobin levels have been associated with an increased risk for non-alcoholic fatty liver disease (NAFLD). Although the mechanism underlying this association is elusive, smoking has been previously related to both higher hemoglobin levels and an increased risk of fibrosis in NAFLD. The present study was conducted to investigate formally the interaction between current smoking, hemoglobin levels, and risk for advanced fibrosis in 433 Turkish patients with biopsy-proven NAFLD.

Method: Preliminary analyses were performed to investigate the existence of substantial statistical interaction between current smoking and hemoglobin levels. Advanced fibrosis was defined as ≥F3 on liver biopsy. Logistic regression models were subsequently implemented to evaluate the effect of current smoking on risk for advanced fibrosis, after adjusting for the effects of age, sex, body mass index, diabetes, and metabolic syndrome.

Results: A significant interaction between current smoking and hemoglobin levels was evident ($p < 0.001$). Advanced fibrosis was identified on liver biopsy in 80 cases, and 84 patients were current smokers. In separate multivariable analyses conducted in the entire cohort and in the subgroups of patients with high and low hemoglobin levels (according to median value in the study cohort: 14.4 g/L), current smoking was associated with increased risk for advanced fibrosis in patients with high hemoglobin (odds ratio 3.32, 95% confidence interval 1.23−7.21, $p < 0.01$) but neither in those with low hemoglobin (odds ratio 0.71, 95% confidence interval 0.28−1.81, $p = 0.52$) nor in the entire study cohort (ratio 1.18, 95% confidence interval 0.73−2.14, $p = 0.79$).

Conclusion: We conclude that hemoglobin is a modifier in the association between current smoking and advanced fibrosis in NAFLD. Our findings suggest that smoking cessation could be a preventive strategy against the progression of fibrosis in NAFLD, particularly for patients with elevated hemoglobin levels.

P06-15YI Ductular reaction predicts the progression of non-alcoholic fatty liver disease

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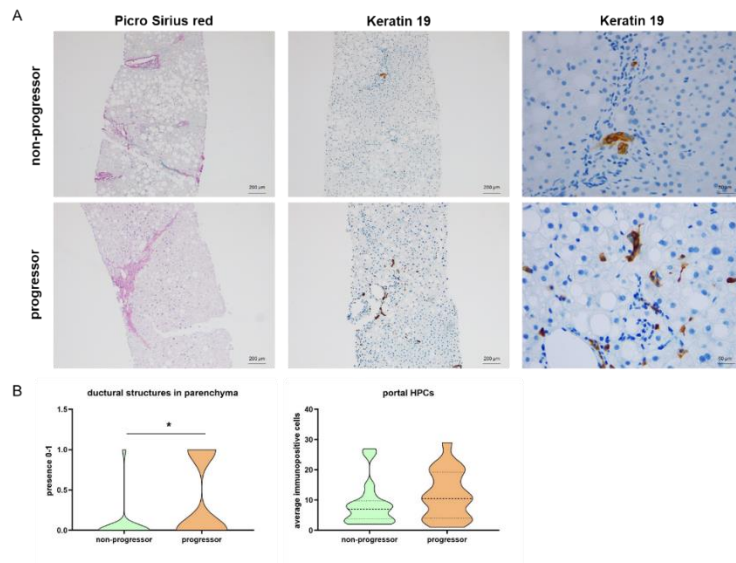Email: ramy.younes@unito.it

Background and aims: Ductular reaction (DR), which comprises an expansion of transit amplifying cells of the terminal branches of the biliary tree, is often seen in advanced NAFLD and is believed to reflect hepatic progenitor cell (HPC) activation. This study aims at investigating the prognostic value of DR/HPCs in NAFLD for NASH development and fibrosis progression.

Methods: We included 36 patients with biopsy-proven NAFLD at Freeman Hospital, Newcastle-Upon-Tyne Hospitals, UK, who underwent at least two liver biopsies more than a year apart. The histological semi-quantitative NAS CRN system was used to score steatosis (S0-3), ballooning (B0-2), lobular inflammation (I0-3) and to stage fibrosis (F0-4). HPCs were quantified in the portal/periportal area and parenchyma based on Keratin 19 immunostaining in the baseline biopsies and correlated with clinicopathological features. Parenchymal ductular cells were scored based on acinar zone topography (1-3) and pattern (single cells, ductular structures). P21 immunostaining was used to identify senescent cells.

Results: In our cohort, the absolute number of periportal HPCs significantly correlated with elevated AST levels ($p < 0.01$). Moreover, the presence of HPCs into the parenchyma correlated with higher AST ($p < 0.01$), ALT ($p < 0.05$) and with the progressive increase of insulin levels ($p < 0.01$). The number of senescent p21-positive hepatocytes predicted advanced fibrosis OR = 1.1 (1.05-1.2, $p = 0.024$) and significantly correlated with GGT and ALP levels (Pearson = 0.376 and 0.421, $p = 0.028$ and $p = 0.015$ respectively). Comparing baseline and follow-up liver biopsies (mean time between biopsies 79 months, range 14-198), the presence of ductular structures in the parenchyma of the baseline biopsy was significantly associated with the progression of at least 1 stage of fibrosis (prevalence of 35% in progressors vs 6% in non-progressors, $p < 0.05$). In addition, a significant correlation with the increase of AST and ALT levels was noted (OR 1.028 and 1.026 respectively). Furthermore, progressors displayed more parenchymal senescence at baseline compared to the non-progressors ($p < 0.01$). In addition, a positive correlation was observed between the presence of parenchymal ductular structures and p21-positive hepatocytes.

Conclusions: In a cohort of biopsy-proven NAFLD patients with follow-up biopsies, HPCs are associated with biochemical signs of inflammation and higher insulin levels. Moreover, the presence of parenchymal ductular structures and parenchymal senescence predict disease progression. Due to the lack of a prognostic biomarker for NAFLD, the development of this tool could acquire paramount importance for both stratifying patients at risk at baseline and the design of targeted therapies to patients with high likelihood of disease progression.

Figure

Figure A: Picro Sirius red and Keratin 19 staining in non-progressor and progressor.

Figure B: ductural structures in parenchyma; portal HPCs.

P06-16YI Coping strategies and fibrosis: a psychological approach to NAFLD

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Background and aims: The coping strategies employed by NAFLD patients have not been studied to date, despite evidence of their relevance in chronic liver pathology, obesity and Type 2 diabetes mellitus (T2DM). This study therefore analyzed whether there are differences in coping strategies used by patients with biopsy-proven NAFLD and obesity or T2DM, depending on the presence or absence of significant fibrosis.

Method: On one hand, a group of 244 patients with obesity, made up of 145 men and 99 women, with a mean age of 55.24 ($SD = 11.46$) was selected. On the other, a group of 158 patients with T2DM, made up of 84 men and 74 women, with a mean age of 59.68 ($SD = 10.02$). Both groups were evaluated by psychosocial interview and The Brief COPE. Fibrosis was classified according to liver biopsy as significant fibrosis ($\geq$F2) or non-significant fibrosis (F0-F1). The independent samples t-test and Cohen´s d were used to compare the coping strategies of the two subgroups.

Results: Statistically significant differences were found in the following subgroups: 1) NAFLD patients with obesity: those who had significant fibrosis compared to those who did not, employed less active coping ($p = 0.005$, $d = 0.382$), self-distraction ($p = 0.034$, $d = 0.282$), positive reframing ($p = 0.011$, $d = 0.339$), acceptance ($p = 0.001$, $d = 0.435$) and humor ($p = 0.005$, $d = 0.370$), and more behavioral disengagement ($p = 0.003$, $d = -0.388$), denial ($p = 0.006$, $d = -0.365$) and self-blame ($p = 0.007$, $d = -0.359$), and 2) NAFLD patients with T2DM: those who had significant fibrosis compared to those did not, used less active coping ($p = 0.000$, $d = 0.583$), instrumental support ($p = 0.048$, $d = 0.338$), positive reframing ($p = 0.020$, $d = 0.391$) and humor ($p = 0.000$, $d = 0.595$), and more behavioral disengagement ($p = 0.000$, $d = -0.577$) and denial ($p = 0.042$, $d = -0.347$).

Conclusion: NAFLD patients with significant fibrosis, whether obese or diabetic, employ maladaptive coping strategies more than NAFLD patients without significant fibrosis.

P06-17YI Network-based drug-repositioning platform identifies pharmacological compounds with anti-steatogenic mode of action

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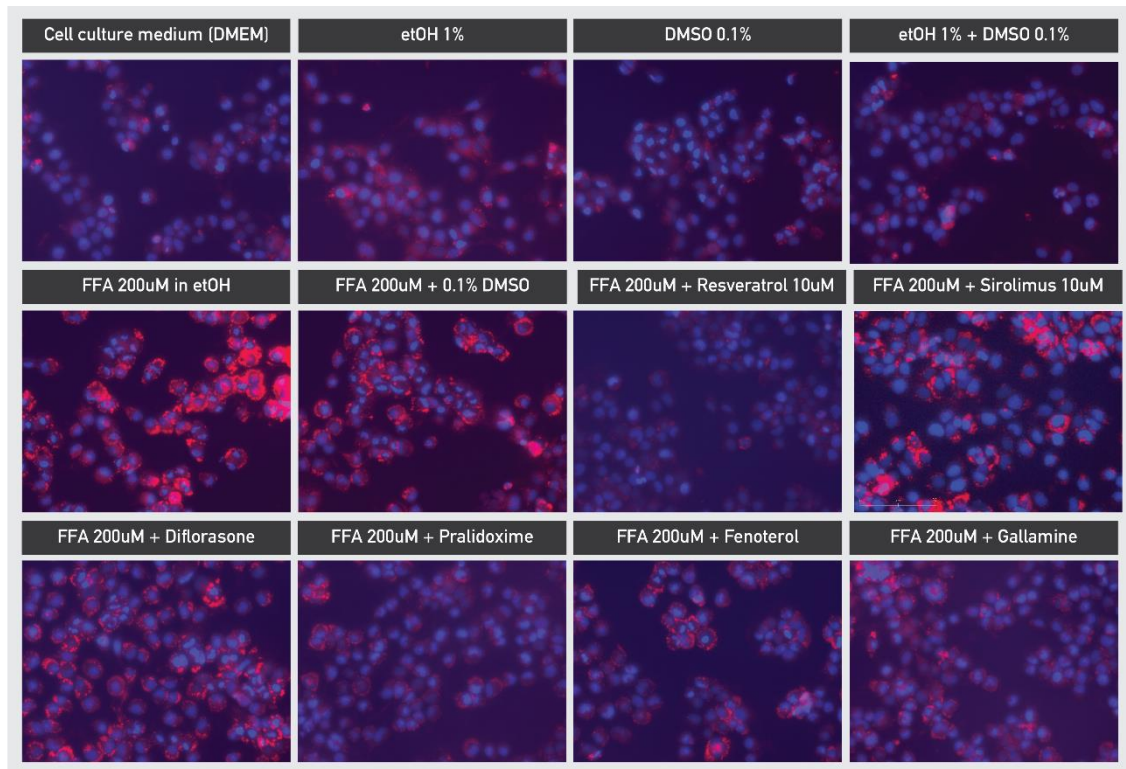Email: danae.zar@gmail.com

Background and aims: Its escalating prevalence in the population (>25% in 2018) is establishing NAFLD as the most common cause of chronic liver disease, however no NAFLD/NASH-specific FDA-approved agent exists. In drug discovery, drug repositioning (DR) grows popular as it is limiting failure, approval time and cost. A DR network-based computational platform was previously developed by our group to suggest compounds for the treatment of NAFLD. The DR platform utilized gene expression datasets from liver biopsies of NAFLD/NASH patients (GEO-NCBI) and datasets derived from compound-treated cell lines (CMap) to construct the respective networks. Via computational comparisons between the drug and NAFLD/NASH-induced networks, compounds affecting NAFLD/NASH steatogenesis were prioritized. Now, complementary in-vitro NAFLD/NASH assays were developed to validate the in-silico predictions and identify novel pro- and anti-steatogenic compounds.

Method: For the DR in vitro screening, hepatocellular cell lines (HuH7, HepG2, Hep3B, FOCUS) were seeded on 384-well plates and exposed to free fatty acids (FFAs-palmitic and oleic acid) 200uM, DR compounds 10uM and FFAs-DR compounds co-treatment for 24h. Intracellular lipid droplets were verified via high-content screening that employed Nile Red fluorescent probe and Hoechst 33342 for nuclei counterstaining. JuLI™Stage (NanoEntek) automatically acquired images to undergo MATLAB analysis. Compounds were found reduce or induce lipid droplet accumulation were examined for their ability to reduce/induce intracellular ROS production using the fluorescent substrate CM-H2DCFDA. Luminex xMAP assays were developed to measure phosphoproteins and cytokine release, while PCA analysis and k-means clustering classification was performed.

Results: The DR platform and in-vitro experiments successfully identified Resveratrol and Rapamycin as possible DR compounds, thus used as positive controls. Gallamine triethiotide, Diflorasone, Fenoterol and Pralidoxime were found to reduce steatosis similar to Resveratrol/Sirolimus. Conversely, Mefloquine, Clomifene and Pimozide were found steatogenic, as droplet formation and ROS production positively correlated to the levels of FFA-treated cells.

Conclusion: Our in-silico approach deduced a network similarity, thus identified compounds both reducing and inducing steatosis in-vitro. A high-throughput setup for NAFLD/NASH drug-screening was developed. Further experiments are necessary to decipher the mechanisms identified compounds facilitate and to assess their in-vivo effects.

Figure:

Cell culture medium (DMEM)	etOH 1%	DMSO 0.1%	etOH 1% + DMSO 0.1%
FFA 200uM in etOH	FFA 200uM + 0.1% DMSO	FFA 200uM + Resveratrol 10uM	FFA 200uM + Sirolimus 10uM
FFA 200uM + Diflorasone	FFA 200uM + Pralidoxime	FFA 200uM + Fenoterol	FFA 200uM + Gallamine

P06-19YI PNPLA3 rs738409 Gene Variant Aggravates Kidney Tubular Injury Among NAFLD Population with Persistent Normal Alanine Aminotransferase

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Background and aims: Rs738409 is associated with kidney function decrease in patients susceptible to non-alcoholic fatty liver disease (NAFLD). While majority of studies are based on NAFLD population with abnormal liver function, those with normal transaminase levels (nALT) are largely ignored. We aimed to ascertain the influence of rs738409 on kidney tubular function in nALT NAFLD patients.

Method: A total of 214 biopsy-proven NAFLD subjects, of which 75 with persistent nALT (ALT <40 U/L for 3 months) were included. Urinary neutrophil gelatinase-associated lipocalin (u-NGAL), a urine biomarker for kidney tubular injury (KTI), was measured. Univariate and multivariate analyses were performed to assess the association between various genotypes and u-NGAL levels.

Results: Between nALT and abnormal transaminase levels (abnALT) NAFLD subjects, those with nALT were older, had lower kidney function and body mass index, while those with abnALT had lower levels of u-NGAL (all p <0.05). For the nALT NAFLD patients, carriers of G/G genotype, as compared to C/C wildtype, exhibited significantly increased u-NGAL level (63.09 ± 53.92 vs. 38.73 ± 18.55, p = 0.028). Similar findings were observed after adjusting for full traditional confounders, in that carriers of the rs738409 G/G genotype was independently associated with higher u-NGAL than those of the wildtype (β coefficient: 24.36, 95%CI: -8.648-26.546, p = 0.044). However, this relationship was not observed in abnALT NAFLD patients.

Conclusion: Carriers of rs738409 G/G in nALT NAFLD are presented with risk for KTI. Coupled by the often overlooked persistent nALT population, such risk is further increased. This suggest NAFLD patients with nALT status should be considered for rs738409 genotyping and carefully monitored for KTI in order to prevent progression of chronic kidney disease.

Figure:

Table 4. Association of *PNPLA3* rs738409 genotype with u-NGAL as categorized by different biopsy-proven NAFLD populations				
Overall	*PNPLA3* rs738409	β coefficient	95% Confidence Interval	*P* value
Model 1	CC	1.00	1.00 - 1.00	
	CG	-1.30	-8.78-6.18	0.733
	GG	-0.24	-8.43-7.95	0.954
Model 2	CC	1.00	1.00 - 1.00	
	CG	0.84	-6.22-7.89	0.815
	GG	1.85	-5.90-9.60	0.64
Model 3	CC	1.00	1.00 - 1.00	
	CG	2.08	-5.37-9.54	0.58
	GG	2.36	-5.80-10.53	0.57
nALT group	*PNPLA3* rs738409	β coefficient	95% Confidence Interval	*P* value
Model 1	CC	1.00	1.00 - 1.00	
	CG	3.913	-12.90 - 20.73	0.644
	GG	24.358	2.65 - 46.06	0.028
Model 2	CC	1.00	1.00 - 1.00	
	CG	8.77	-6.85 - 24.38	0.266
	GG	25.60	5.11 - 46.10	0.015
Model 3	CC	1.00	1.00 - 1.00	
	CG	8.11	-9.00 – 25.24	0.312
	GG	22.59	-0.29 – 44.88	0.047
abnALT group	*PNPLA3* rs738409	β coefficient	95% Confidence Interval	*P* value
Model 1	CC	1.00	1.00 - 1.00	
	CG	3.91	-12.90 - 20.73	0.644
	GG	24.36	2.65 - 46.06	0.028
Model 2	CC	1.00	1.00 - 1.00	
	CG	-2.97	-8.60 - 2.66	0.299
	GG	-5.55	-11.46 - 0.36	0.065
Model 3	CC	1.00	1.00 - 1.00	
	CG	-2.43	-8.30 - 3.45	0.415
	GG	-4.41	-10.57 -1.75	0.159
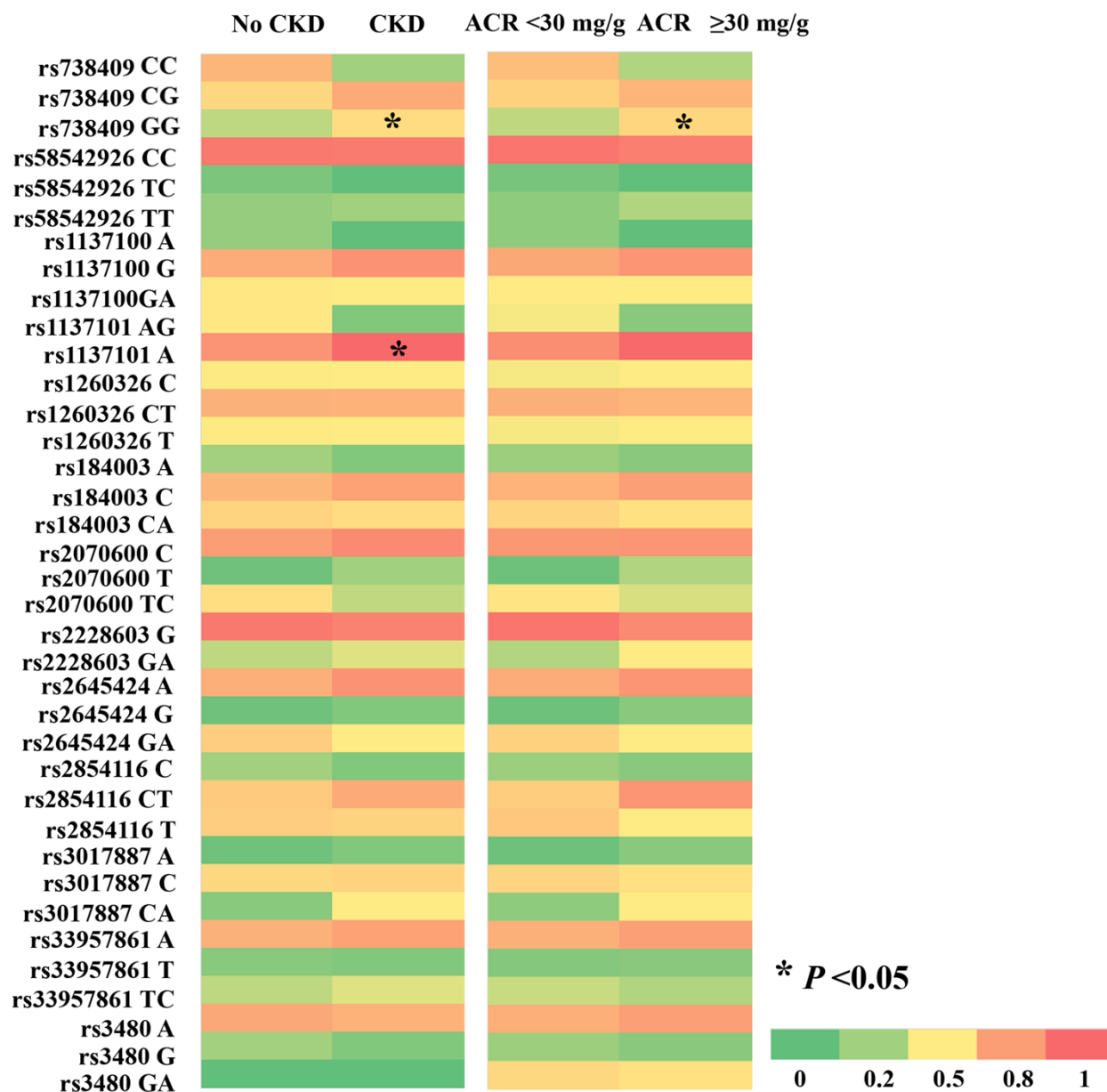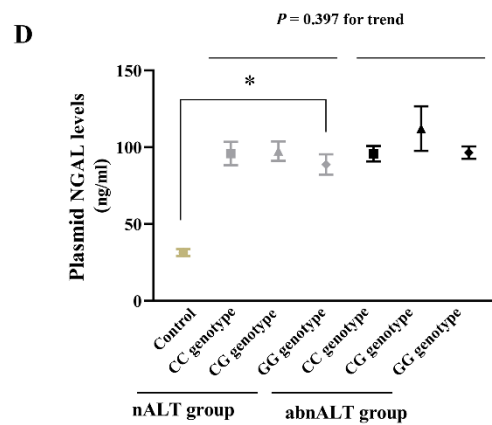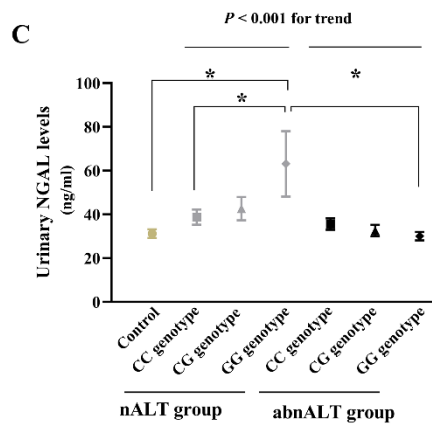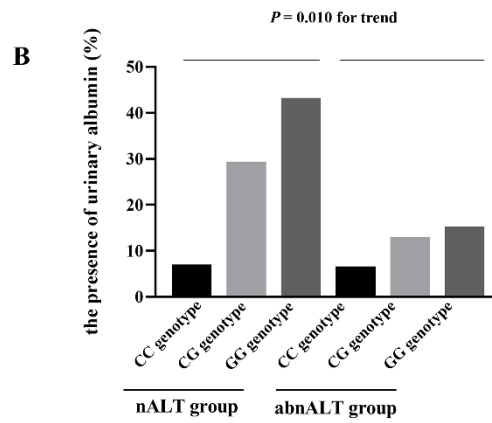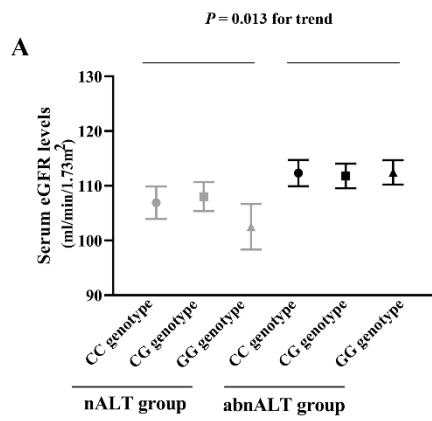
P06-20 Myeloid cells-derived osteopontin protects from diet-induced non-alcoholic fatty liver disease in mice

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Background and aims: Osteopontin (OPN) is an extracellular cytokine that is up-regulated in patients with non-alcoholic steatohepatitis (NASH). Myeloid cells show high expression of OPN in mouse models of NASH. Previous studies indicated that OPN promotes an anti-inflammatory phenotype; however, the mechanism driving these effects remained to be elucidated. Therefore, we hypothesized that overexpression of OPN in myeloid cells could protect from NASH.

Method: Mice overexpressing OPN in myeloid cells (*Opn*^Mye Tg) were generated using *Opn-Stop*^fl/fl and recombination with *Lyz2.Cre* mice. *Cre*+ littermates were used as control littermates. Mice were fed up to 6 months with either a western diet mimicking NASH (40% of calories from fat, 20% from fructose and 1.8% (w/w) cholesterol) or an isocaloric control diet. Samples were collected for histological assessment, analysis of biochemical parameters and RNA sequencing.

Results: Control littermates fed with the NASH-inducing diet developed key features of NASH including steatosis, hepatocyte ballooning degeneration, inflammation and chicken-wire fibrosis. These events were more prominent in male than in female mice. However, *Opn*^Mye Tg mice were fully protected from NASH, as shown by a significant reduction in hepatic triglycerides, cholesterol, immune cell infiltration and pro-fibrogenic signals. Amelioration of NASH was more dramatic in male than female *Opn*^Mye Tg mice; yet, female mice were protected from insulin resistance. Transcriptome analysis revealed that *Opn*^Mye Tg had a significant reduction in the hepatic expression of genes involved in fatty acid transport, beta-oxidation and cholesterol biosynthesis. *Opn*^Mye Tg mice presented upregulation of the urea cycle. Notably, myeloid cells isolated from these mice displayed a significant increase in the expression of arginase 2 (Arg2) protein, which correlated with Opn and was also associated with down-regulation of nitric oxide synthetase 2 *(Nos2 or iNos)*, *Tnf* and *Il1b mRNAs*.

Conclusion: *Opn* in myeloid cells regulates hepatic fatty acid transport, cholesterol biosynthesis and the urea cycle. Moreover, *Opn* regulates expression of *Arg2* in myeloid cells and reduces the pro-inflammatory signaling. Overall, these events contribute to protection from NASH.

P06-21 Intervention with HE-700 reduces the development of liver inflammation in obese HFD-treated Ldlr-/-.Leiden mice by ameliorating the build-up of hepatic cholesterol

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a complex multifactorial disorder that is characterized by dysfunction of hepatic lipid metabolism and, related to that, chronic inflammation in the liver. NAFLD has become the most common cause of chronic liver disease in many countries, and its prevalence continues to rise in parallel with increasing rates of obesity. In the present study we evaluated the putative NAFLD-attenuating effects of a multicomponent medicinal product consisting of natural ingredients: HE-700

Method: Ldlr-/-.Leiden mice were fed a high-fat diet (HFD) for 24 weeks to induce obesity-associated metabolic dysfunction including hepatic steatosis and inflammation. HE-700 or vehicle control (saline) was intraperitoneally administered 3 times weekly at 1.5 ml/kg for the last 18 weeks of the study. Liver and adipose tissue histology was performed in combination with extensive hepatic gene expression analysis (next generation sequencing), the results of which were substantiated with immunohistochemistry and liver lipid analysis.

Results: Treatment with HE-700 did not affect body weight, plasma lipids, glucose and insulin, adipose tissue and liver weight, and total liver steatosis. Histopathological analysis of inflammatory aggregates indicated reduced lobular inflammation after HE-700 treatment, which was associated with modulation of genes involved in control of lipid processing (e.g. *Abcg8* and *Pla2g4c*) and the inflammatory response including *Socs3*, *Gzma* and the neutrophil-attracting chemokine *Cxcl1*. More refined immunohistochemical analysis of neutrophils, indeed, confirmed an effect on neutrophils and revealed a significant reduction of neutrophil aggregates induced by HE-700. Furthermore, upstream regulator analysis predicted that HE-700 treatment affected the liver metabolite 'cholesterol' based on the observed gene expression changes associated with cholesterol handling. Subsequent biochemical analysis substantiated this prediction and showed that HE-700 significantly reduced HFD-induced free cholesterol levels in the liver and tended to lower cholesteryl esters while triglyceride concentrations remained unchanged. Intrahepatic free cholesterol levels were found to correlate significantly with hepatic inflammation (number of inflammatory aggregates), thereby providing a potential rationale for the observed anti-inflammatory effects of HE-700.

Conclusion: HE-700 treatment has anti-inflammatory molecular (e.g. *Cxcl1*) and cellular (e.g. neutrophil content) effects in obese Ldlr-/-.Leiden mice with NASH. A reduction of lipotoxic lipid species, i.e. free cholesterol, may provide a rationale for the observed NASH-attenuating effect.

P06-22 A preclinical study on the effect of an oral edaravone formula on NASH in a rabbit model

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Background and aims: Non-alcoholic steatohepatitis (NASH) is one of chronic diseases that affects a large population worldwide. Despite of efforts made by a number of pharmaceutical companies in developing therapeutic drugs for NASH, no effective drug passes phase 3 clinical trials and is in the market. Thus, developing an effective drug to treat non-alcoholic fatty liver disease (NAFLD) and NASH has become a challenge for both academics and industry. It is known that oxidative stress is critical in the pathogenesis of NASH, therefore we hypothesized that the free radical scavenger edaravone could have a therapeutic value in NASH. The aim of this study is to test the hypothesis in a rabbit model.

Method: Twenty-five New Zealand male rabbits, 2 months old, were used for this study. Basal information about body weight and biochemical parameters were collected on all rabbits before experiments. Five rabbits were used as a negative normal control (group 1). The rest 20 rabbits were used for the induction of NASH by high fat and high cholesterol diet (100 gram/day) feeding for a period of 8 weeks. Four weeks after induction, one group of rabbits (group 2, n = 5) were culled as a basal line NASH control and the rest NASH rabbits were divided into 3 groups: Group 3: None treated negative control; Group 4: treated with ASK1 inhibitor (Selonsertib) GS-4997 at 6 mg/kg/day orally; and Group 5: treated with edaravone oral formulation at 15 mg/kg/day. After the trial, all internal organs were collected for histology and biochemistry investigations.

Results: All rabbits reached the end point without any mortality. Blood sugar level in non-treated Group 3 was increased reaching the diabetic level, but were normal in both Group 4 and 5. The serum activities of AST and ALT in Group 4 and 5 were reduced compared to the non-treated group. Histology showed NAS scores, inflammatory scores, hepatocyte steatosis score and hepatocyte ballooning scores were significantly reduced in both GS-4997 group and edaravone group and these scores were comparable between GS-4997 and edaravone groups without significant difference. Most strikingly, the fibrosis score in edaravone group was substantially reduced compared with GS-4997 group. Comparing with non-treated group, the fibrosis score was reduced by 33.6% in GS-4997 and 68.5% in edaravone groups respectively.

Conclusion: The data support our hypothesis that oxidative stress is a critical pathogenic factor regulating NASH development. The antioxidant edaravone can reduce the inflammation, abnormal lipid metabolism, serum ALT and AST levels and liver fibrosis in the rabbit model of NASH. Our results also support further clinical trials of oral edaravone to treat NAFLD and NASH.
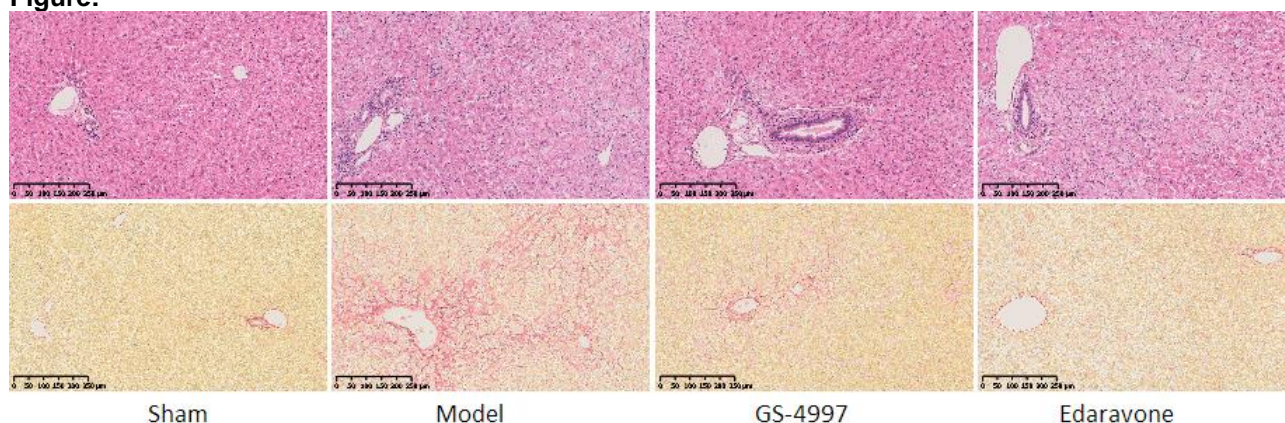
Figure:

Sham   Model   GS-4997   Edaravone

Figure 1 shows the significant reduction in inflammation and fibrosis in rabbits treated with GS-4997 or oral edaravone formulation.

INDUSTRY

Industry satellite symposia

ALLERGAN PHARMACEUTICALS INTERNATIONAL Ltd

Thursday 26 September 2019, 18:30-19:30 – Room: España 5

Targeting NASH: exploring a complex disease and where we are heading
Chair Frank Tacke, *Germany*

18:30 - 18:35	Welcome and introduction Frank Tacke, *Germany*
18:35 - 18:50	A brief (natural) history of NASH: steatosis, inflammation and fibrosis Frank Tacke, *Germany*
18:50 - 19:05	Fibrosis severity: what does it mean for patients? Fabio Marra, *Italy*
19:05 - 19:20	Treating NASH: what does the future look like? Salvador Augustin, *Spain*
19:20 - 19:30	Audience Q&A/panel discussion and meeting close Frank Tacke, *Germany* Panelists: Fabio Marra, *Italy* - Salvador Augustin, *Spain*

Objectives:

- To enable scientific discussion around NASH clinical/histological targets (e.g. steatosis vs inflammation vs fibrosis.
- To discuss the relevance of NASH fibrosis severity with a focus on:
    o Link to outcomes;
    o Which patients are likely to progress?
- Based on what we currently know about NASH and learnings from other diseases, assess where the field is currently and what future treatment strategies might look like.

INTERCEPT

Friday 27 September 2019, 13:00-14:00 – Room: España 5

NASH: Bringing the evidence into practice
Chair : Jean-François Dufour, *Switzerland*

13:00 - 13:05   Welcome and introductions
                       Jean-François Dufour, *Switzerland*

13:05 - 13:25   Evaluation of NAFLD severity: What does good practice look like now, and
                       in the future?
                       Pierre Bedossa, *France*
                       Jerome Boursier, *France*

13:25 - 13:40
                       What does efficacy of intervention look like in practice?
                       Elisabetta Bugianesi, *Italy*
13:40 - 13:55
                       Implications of clinical trial data for day-to-day management
                       Jean-François Dufour, *Switzerland*
13:55 – 14:00
                       Q&A and meeting close
                       Jean-François Dufour, *Switzerland*
                       Panelists: Pierre Bedossa, *France* - Jerome Boursier, *France* - Elisabetta
                       Bugianesi, *Italy*

Company profiles

AIFORIA

Tukholmankatu 8
00290 Helsinki
Finland

www.aiforia.com

Aiforia is a Helsinki, Finland based start-up enabling medical professionals to have access to deep learning AI for image analysis through a cloud-based software. Guided by Aiforia, users can build their own AI models to automate a variety of image analysis tasks. No coding or local hardware is needed.

ALLERGAN PHARMACEUTICALS INTERNATIONAL Ltd

5 Giralda Farms
07940 Madison, NJ
United States

www.allergan.com

Allergan plc (NYSE: AGN), is a bold, global pharmaceutical company focused on developing, manufacturing and commercializing branded pharmaceuticals, devices and biologic products for patients around the world.

For more information, visit Allergan's website at www.Allergan.com.

APOLLO ENDOSURGERY

Unit 10 Grimbald Crag Court
St James Business Park
HG5 8QB Knaresborough
United Kingdom

www.apolloendo.com

Apollo Endosurgery, Inc. is a medical device company focused on less invasive therapies for the treatment of obesity as well as other gastrointestinal conditions. Apollo's device-based therapies are an alternative to invasive surgical procedures, thus lowering complication rates and reducing total healthcare costs. Apollo's portfolio includes OVERSTITCH™ and ORBERA365™

BOEHRINGER INGELHEIM INTERNATIONAL GmbH

Binger Strasse 173
55216 Ingelheim am Rhein
Germany

www.boehringer-ingelheim.com

Innovative medicines for humans and animals have for more than 130 years been what the research-driven pharmaceutical company Boehringer Ingelheim stands for. Boehringer Ingelheim is one of the pharmaceutical industry's top 20 companies and to this day remains family-owned. Day by day, some 50,000 employees create value through innovation for the three business areas human pharmaceuticals, animal health and biopharmaceutical contract manufacturing.

ECHOSENS

30, place d'Italie
75013 Paris
France

www.echosens.com

In 2003, Echosens significantly changed the practice of liver diagnosis with FibroScan®, the unique device using patented and validated VCTE™ for liver stiffness assessment, and CAP™ for steatosis quantification.
FibroScan® device is recognized worldwide as the reference for non invasive liver diagnosis with more than 2,000 medical publications and 40 guidelines recommendations.
We are working hand in hand with physicians, medical academic research, pharmaceutical companies for patient identification and monitoring in NAFLD/NASH.
As patient education is a major issue, we have created the "myliverexam" website that provides information about the role of the liver and why it is vital to the global health.

ENDRA LIFE SCIENCES

3600 Green Court, Suite 350
48105-1570 Ann Arbor
United States

www.endrainc.com

ENDRA Life Sciences (NASDAQ: NDRA) is a development stage company with a breakthrough Thermo-Acoustic Enhanced UltraSound (TAEUS) enabling clinicians to visualize human tissue composition, function and temperature. Initial application is quantification of fat in the liver, for early detection and monitoring of NAFLD, which affects over 1 billion people globally.

KEYWORDS: NAFLD, Fatty liver disease, Therm-Acoustic Enhanced Ultrasound

GENFIT SA

885 avenue Eugène Avinée
59120 Loos
France

www.genfit.com

GENFIT, a biopharmaceutical company focused on discovering and developing therapeutic and diagnostic solutions in metabolic/liver diseases, is evaluating elafibranor in a P3 trial in NASH as well as in PBC. GENFIT is also developing NIS4™, a novel blood-based in vitro diagnostic test to easily identify NASH patients.

GILEAD SCIENCES EUROPE LTD

2 Roundwood Avenue, Stockley Park
UB11 1AF Uxbridge
United Kingdom

www.gilead.com

Gilead Sciences, Inc. is a research-based biopharmaceutical company that discovers, develops and commercializes innovative medicines in areas of unmet medical need. For 20 years Gilead has been working to transform the treatment of liver diseases.

Gilead is advancing our understanding of NASH, including: disease progression, diagnosis and potential future therapies.

INSPHERO
Wagistrasse 27
8952 Schlieren
Switzerland

www.insphero.com

InSphero is the pioneer of scalable, 3D-cell-based assay solutions and organ-on-a-chip technology for drug discovery and safety testing. Through partnerships, InSphero supports pharma and biotech researchers in successful decision-making by accurately recapitulating healthy and diseased human physiology in vitro.

INTERCEPT

Two Pancras Square
N1C 4AG London
United Kingdom

https://www.interceptpharma.com

Intercept is a biopharmaceutical company focused on the development and commercialization of novel therapeutics to treat progressive non-viral liver diseases, including primary biliary cholangitis (PBC), nonalcoholic steatohepatitis (NASH), primary sclerosing cholangitis (PSC) and biliary atresia. Founded in 2002 in New York, Intercept now has operations in the United States, Europe and Canada.

MSD

200 Galloping Hill Road
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www.msd.com

For more than a century, MSD has been inventing for life, bringing forward medicines and vaccines for many of the world's most challenging diseases. Today, MSD continues to be at the forefront of research to deliver innovative health solutions and advance the prevention and treatment of diseases that threaten people and animals around the world - including cancer, cardio-metabolic diseases, emerging animal diseases, Alzheimer's disease and infectious diseases including HIV and Ebola. For more information, visit www.msd.com and connect with us on Twitter, LinkedIn and YouTube.

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VLV BIO

Hästholmsvägen 32
13130 Nacka
Sweden

www.vlvbio.com

At VLVbio, we offer unique assays for accurate and non-invasive detection of liver damage and disease.

Our biomarker assays, M30® and M65®, measure apoptosis and total cell death respectively, and are used globally within academia, industry and healthcare sectors.

Measuring apoptosis and levels of total cell death is of interest in the diagnosis and treatment follow-up of patients suffering from liver diseases such as NASH and ASH, in the development of new drugs, and when assessing toxicological effects of pharmaceuticals and other substances.

Join us at our booth and let us discuss more about our biomarker assays!

ACKNOWLEDGEMENTS

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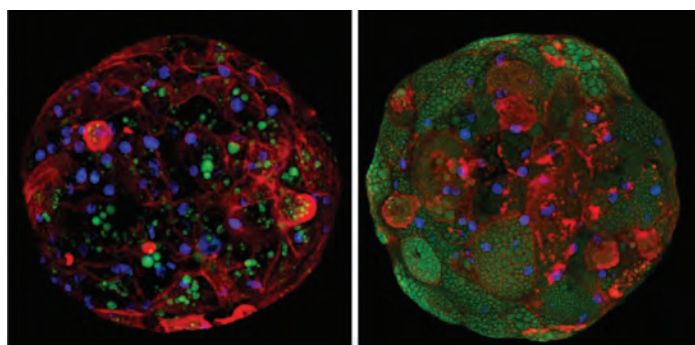
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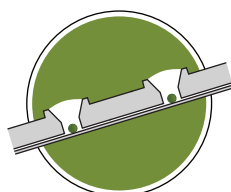*Staining: Lipids (green), nuclei (blue), and plasma membrane (red).*

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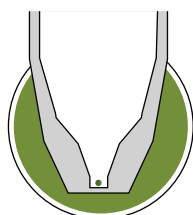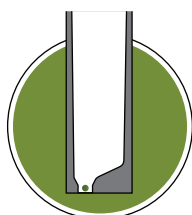
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- Attend ePosters P05-12 and P06-05 to review exciting data generated with our NASH model
- Visit Booth 11 to discuss your NAFLD research projects with our liver disease platform team
- Learn more about NAFLD & NASH solutions online at insphero.com/event/nafld2019/

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TARGETING NASH

Exploring a complex disease and where we are heading

Congress Centre: Barceló Sevilla Renacimiento
18:30–19:30, 26th September, Seville
Room: Espana 5

Time	Session	Speaker
18:30–18:35	Welcome and introduction	Frank Tacke (Chair) University Hospital Aachen, Germany
18:35–18:50	A brief (natural) history of NASH: steatosis, inflammation and fibrosis	Frank Tacke
18:50–19:05	Fibrosis severity: what does it mean for patients?	Fabio Marra University of Florence, Italy
19:05–19:20	Treating NASH: what does the future look like?	Salvador Augustin Institute de Recerca Vall d'Hebron, Barcelona, Spain
19:20–19:30	Audience Q&A/panel discussion and meeting close	Frank Tacke/All

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NASH

BRINGING THE EVIDENCE INTO PRACTICE

FRIDAY, 27TH SEPTEMBER 2019
13:00–14:00

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