



EASL[™] NAFLD SUMMIT 2022

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The pivotal meeting on fatty liver disease

ABSTRACT BOOK

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**POSTER
ABSTRACT
PRESENTATION**

BASIC SCIENCE

P01-01 Single-nucleotide polymorphisms in the DNA repair genes affect the risk of non-alcoholic fatty liver disease occurrence

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) mechanism is related to the accumulation of free fatty acids, inflammation and oxidative stress. They may result in DNA damage, which requires an appropriate DNA damage response. The major pathway responsible for oxidative DNA damage repair is base excision repair (BER). We have decided to study the associations between NAFLD and single-nucleotide polymorphisms (SNPs) in genes involved in BER: nei like DNA glycosylase 1 (*NEIL1*), exonuclease G (*EXOG*), endonuclease G (*ENDOG*) and DNA polymerase gamma (*POLG*). This study was funded by the National Science Centre of Poland (No. 2019/35/O/NZ5/02502).

Method: In this study NAFLD patients and healthy controls were participated. We selected five SNPs: rs4462560 (*NEIL1*), rs1065800 (*EXOG*), rs9838614 (*EXOG*), rs2977998 (*ENDOG*), rs1054875 (*POLG*). DNA was isolated from the whole blood and the genotyping was performed using TaqMan probes. The results were calculated as odds ratios (ORs) with 95% confidence intervals.

Results: The obtained findings showed that the following genotypes and alleles increase the risk of NAFLD occurrence: genotype C/G of rs4462560 (*NEIL1*), genotype A/A and allele A of rs1065800 (*EXOG*), genotypes G/G and T/T of rs9838614 (*EXOG*), genotypes C/C, C/T and allele C of rs2977998 (*ENDOG*) and genotype A/A and allele A of rs1054875 (*POLG*). On the other hand, the following ones decreases the risk of NAFLD: allele G of rs1065800 (*EXOG*), genotype G/T of rs9838614 (*EXOG*), genotype T/T and allele T of rs2977998 (*ENDOG*) and genotype A/T and allele T of rs1054875 (*POLG*). The findings are presented in the table below.

Conclusion: The findings suggest that selected variants in the genes involved in BER may modulate the risk of the NAFLD occurrence. We suspect that the other genes of BER can influence the development of the fatty liver disease.

Figure: The risk of NAFLD occurrence presented as odds ratios (ORs) with 95% confidence intervals (CI).

Genotypes/ Alleles	ORs (5-95% CI)	p value	Genotypes/ Alleles	ORs (5-95% CI)	p value
NEIL1 rs4462560			ENDOG rs2977998		
CC	0.678 (0.453-1.014)	0.058	CC	9.684 (3.700-25.344)	<0.001
CG	1.671 (1.113-2.508)	0.013	CT	2.839 (1.625-4.960)	<0.001
GG	0.001 (0.000-+inf)	0.989	TT	0.022 (0.008-0.060)	<0.001
C	0.792 (0.546-1.149)	0.219	C	13.897 (6.928-27.878)	<0.001
G	1.262 (0.871-1.831)	0.219	T	0.072 (0.036-0.144)	<0.001
EXOG rs1065800			POLG rs1054875		
AA	2.423 (1.285-4.568)	0.006	AA	75.982 (10.310-559.948)	<0.001
AG	0.849 (0.541-1.333)	0.478	AT	0.258 (0.159-0.420)	<0.001
GG	0.641 (0.365-1.123)	0.120	TT	11.080 (0.615-1.898)	0.789
A	1.726 (1.167-2.552)	0.006	A	2.692 (1.728-4.194)	<0.001
G	0.579 (0.392-0.857)	0.006	T	0.389 (0.251-0.602)	<0.001
EXOG rs9838614					
GG	13.693 (4.021-46.623)	<0.001			
GT	0.209 (0.127-0.343)	<0.001			
TT	2.779 (1.628-4.745)	<0.001			
G	0.929 (0.605-1.426)	0.735			
T	1.077 (0.701-1.654)	0.735			

P01-04 Effects of high fat diet on development of NASH in the NIF mouse model

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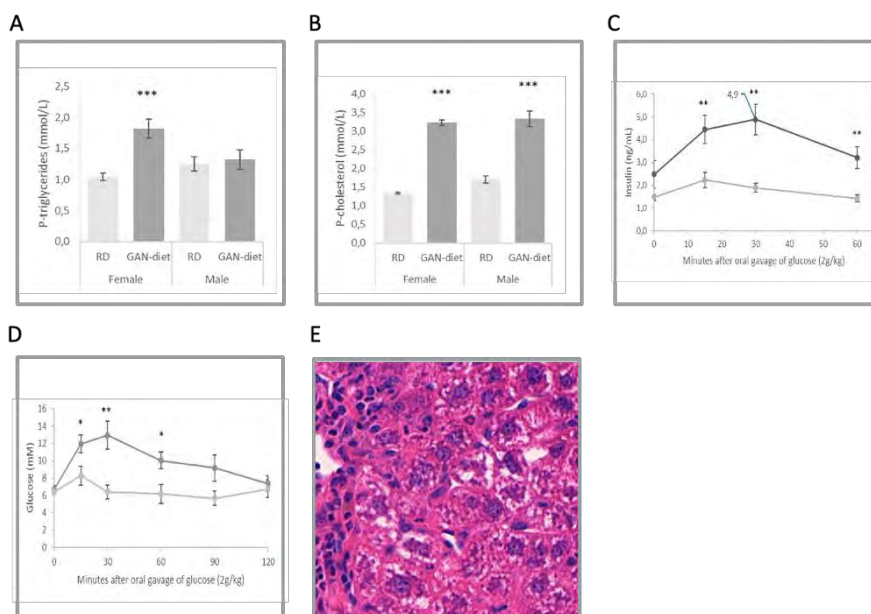
Background and aims: Inflammation in the absence of pathogens occurs in all tissues in response to a wide range of stimuli that cause tissue stress and injury. Such sterile inflammation (SI) is a key process in several liver conditions including in non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH). We have recently reported on a novel mouse model for sterile liver inflammation, the NIF mouse. In this model we showed how plasticity in NKT cells can drive an initial type 1 inflammatory response and promote the transition into a type 2 inflammatory response and overlapping key cellular and molecular events characterizing human liver diseases such as e.g. NAFLD/NASH. Here we fed the NIF mouse GAN diet to elucidate how the model would respond to a diet known to induce steatosis and many characteristics of NAFLD/NASH in other genetic backgrounds.

Method: NIF mice on GAN diet and standard diet (SD) for 3, 5 and 9 weeks starting at 3 weeks of age were analysed for markers of metabolic stress, inflammation and fibrosis. Before end point, an oral glucose tolerance test (OGTT) was done on fasting mice and samples for measurement of glucose-stimulated insulin secretion (GSIS) was taken. At end point livers were collected, sectioned and stained with HandE and Picro Sirius Red (PSR).

Results: GAN diet was found to rapidly induce metabolic stress evident by markers such as an increase in triglycerides, cholesterol and GSIS after 3 weeks on the diet (Figure 1 A- C). After 9 weeks on GAN diet we also observed an impaired OGTT (Figure 1 D) and hepatocyte damage visual as hepatocyte ballooning (Figure 1 E).

Conclusion: NIF mice on GAN diet show signs of metabolic stress and hepatocyte damage in addition to the spontaneous chronic inflammation and fibrosis observed in NIF mice fed SD. This, together with the robust fibrotic component, early on-set, spontaneous nature, 100% reproducibility, and similarity to human NASH makes the NIF model a unique tool to separate treatment effects of new drugs mediated by immunological versus metabolic pathways.

Figure:



P01-05-YI Hydroxysteroid 17-beta sterol dehydrogenase 13 knockdown improves liver steatosis in mice fed a high-fed diet

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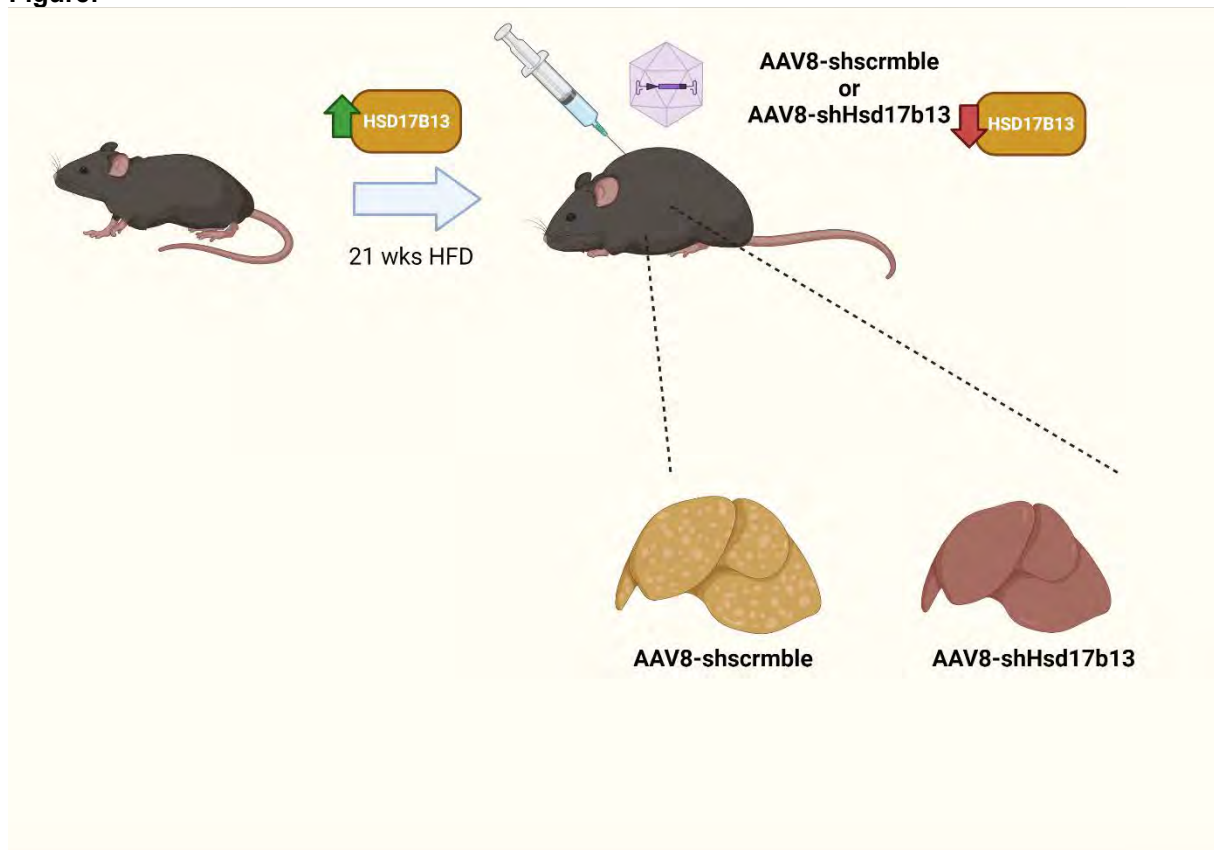
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Background and aims: Non-alcoholic fatty liver disease (NAFLD) associated with obesity has become a global burden with no effective therapeutic to directly target it. Hydroxysteroid 17-Beta Dehydrogenase 13 (HSD17B13) is a lipid-droplet associated protein identified to play a role in various stages of NAFLD including elevated levels in models of simple steatosis. Some loss of function variants have also been identified to play a protective role however the mechanism behind this is unknown.

Method: NAFLD was induced in male C57/BL6 mice were fed a high fat (45% kCal) diet for 21 weeks before administration of either shscrambl (n = 8) or shHsd17b13 (n = 8) AAV8-virus for Hsd17b13 knockdown. Chow-fed mice (n = 8) were used as controls. Liver histology, triglycerides and serum ALT levels were determined to assess liver health after 2 weeks. RT-qPCR was used to determine gene expression of molecular pathways.

Results: There was no effect of shHsd17b13 knockdown on body weight, fat composition or glycaemia levels. Liver triglyceride levels were decreased by ~25% in shHsd17b13 knockdown mice and liver histology also showed improvement in steatosis, by preventing hepatocyte ballooning. Serum ALT levels were greatly reduced with shHsd17b13. Gene expression of fatty acid uptake markers were elevated in shscrambl mice and rescued to levels close to chow-fed controls whilst fibrosis markers trended to improve. Other markers involved in lipid metabolism, ER stress, autophagy and retinoic acid metabolism were not altered by shHsd17b13knockdown.

Conclusion: *Hsd17b13* knockdown was able to alleviate NAFLD in mice fed a long term HFD. Changes in fatty acid uptake may contribute to the mechanism however more investigation is needed to confirm the complete molecular pathway by which Hsd17b13 can exert its beneficial effect. Thus, we confirm Hsd17b13 to be important in the development of NAFLD and establishing it as a strong therapeutic target.

Figure:

P01-08 LIVRQNac increases fatty acid oxidation in a primary human hepatocyte model of non-alcoholic steatohepatitis

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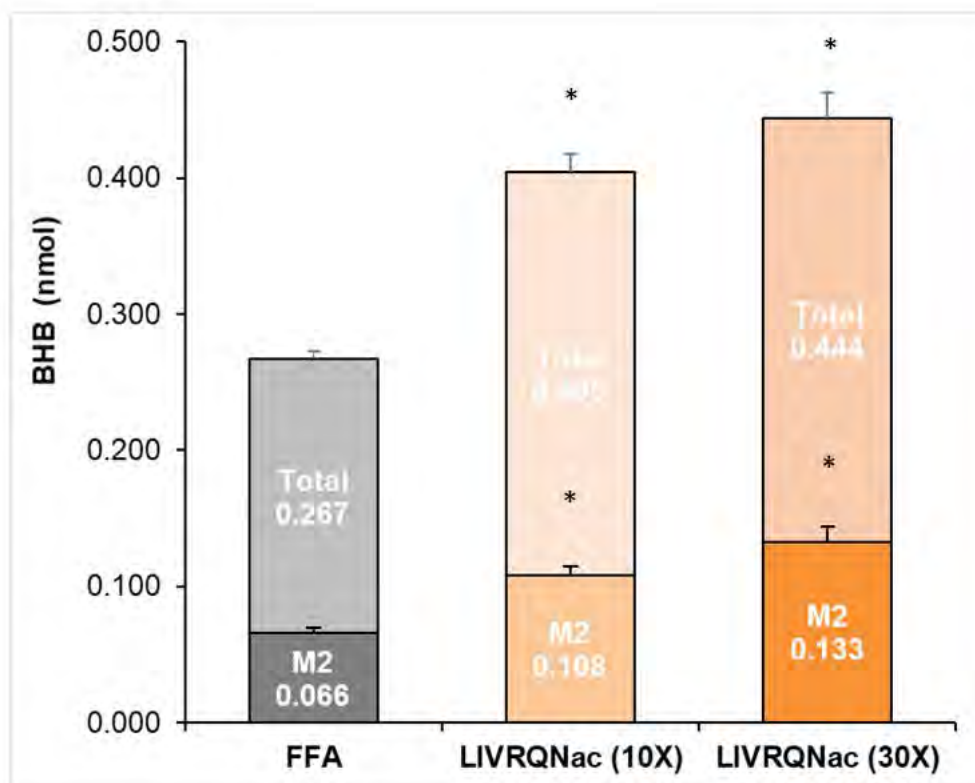
Background and aims: Endogenous metabolic modulators (EMM) are naturally occurring compounds with signaling and regulatory properties. When selectively combined, in unique stoichiometric ratios, EMMs can treat complex diseases with system-wide dysregulation of metabolic pathways. LIVRQNac, an EMM composition of 5 amino acids (AA) and n-acetylcysteine (Nac), decreased liver fat accumulation (*in vivo* and *in vitro*) by reducing triglyceride accumulation. AXA1125, the clinical formulation of these same EMMs, decreased liver fat content in subjects with non-alcoholic fatty liver disease by magnetic resonance imaging measurement of liver proton density fat fraction, MRI-PDFF. The present study investigated the *in vitro* dose-dependent effects of LIVRQNac on fatty acid oxidation (FAO), the putative mechanism of these effects, in a primary human hepatocyte (PHH) NASH model.

Method: PHHs were plated on collagen coated wells and incubated with 5% CO₂. On day 3, cells were incubated in custom media containing 500 µM carnitine, 10 µg/ml insulin, EGF, 1 µM dexamethasone, and custom AA concentrations (as in healthy human plasma) with and without LIVRQNac (10X and 30X) and with lipotoxic insult (saturated free fatty acids [2:1 oleate: palmitate] and tumor necrosis factor alpha (1 ng/ml). After 24 hours, cells were treated for 1 hour with medium containing LIVRQNac and lipotoxic insult with [U-¹³C] palmitate replacing palmitate and then analyzed for ¹³C-labeled palmitoylcarnitine, acetylcarnitine, acetyl-co-enzyme A, β-hydroxybutyrate, and other metabolites using liquid and gas chromatography-mass spectrometry.

Results: ¹³C labeling of fatty acid-derived metabolites (%) was significantly increased by LIVRQNac treatment vs control. Increases were observed in labeled palmitoylcarnitine ($p < 0.001$), acetylcarnitine ($p < 0.05$), and acetyl-CoA ($p < 0.05$) in LIVRQNac- 10X, and 30X treated cells vs control. M2 labelled β-hydroxybutyric acid (terminal FAO product) increased 102% in LIVRQNac-treated cells (0.108 nmol, 10X; 0.133 nmol, 30X) vs control (0.066 nmol; $p < 0.0001$; Fig).

Conclusion: Increases in labeled palmitoylcarnitine, acetylcarnitine and acetyl-coA reflected an increase in FAO in PHHs treated with LIVRQNac. Consistently, there was an increase in total and labelled β-hydroxybutyric acid in LIVRQNac-treated cells indicating an increase in ketogenesis. These data support a mechanism for AXA1125's clinical effect of decreasing liver fat, by increasing ketogenesis and increasing FAO.

Figure: Increases (%) in labeling of the terminal FAO metabolite BHB, derived from [U-¹³C] palmitate tracer, and total pool size are indicative of the dose-dependent increase in FAO with LIVRQNac



*p < 0.0001, analysis of variance; BHB, the ketone body, β -hydroxybutyrate; FAO, fatty acid oxidation; M2, mass isotopomers where number represents the number of heavy atoms in the molecule

P01-12-YI The role of Gremlin-1 in pathogenesis and treatment in rodent and human NASH

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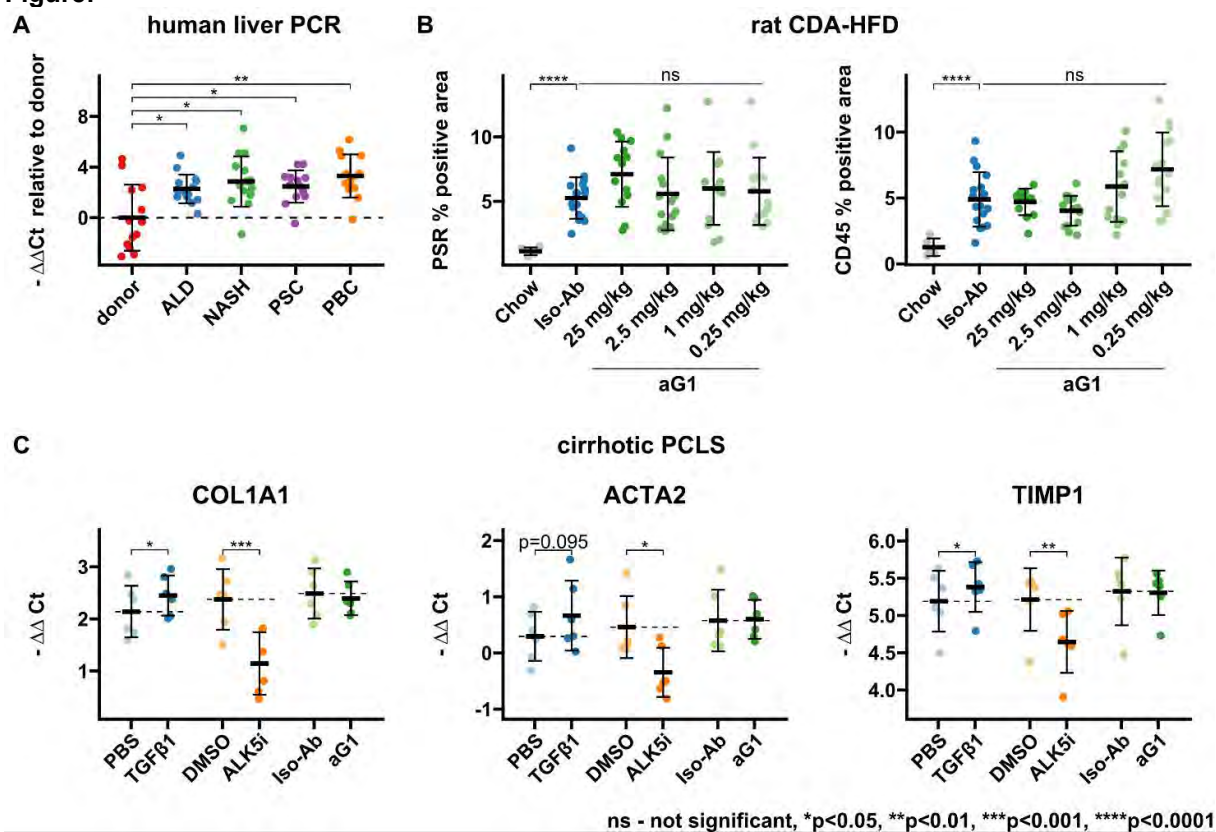
Background and aims: Gremlin-1 (GREM1) belongs to the superfamily of cystine knot proteins and has been implicated in organ fibrosis via inhibition of bone-morphogenetic protein (BMP) signaling. It is upregulated in hepatic stellate cells (HSCs) upon *in vitro* activation, and liver tissue expression increases in non-alcoholic steatohepatitis (NASH) commensurate to the degree of fibrosis. We studied the expression and role of GREM1 in the pathogenesis of rodent and human NASH inflammation and fibrosis.

Method: Gene expression was assessed by qPCR and RNAscope ISH in human and rodent healthy and NASH liver. LX2 cells and primary human hepatic myofibroblasts were used for testing anti-GREM1 antibody (aG1-Ab) *in vitro*. Male Sprague Dawley rats aged 8-10 weeks were fed a 0.1% methionine-, choline-deficient high fat diet (CDA-HFD) for 12 weeks and from week 6 were treated weekly with subcutaneous aG1-Ab or isotype control (iso-Ab). Liver fibrosis and inflammation were assessed by picosirius red (PSR) and CD45 immunostaining, respectively. Precision-cut liver slices (PCLS) were prepared from human cirrhotic explant livers and treated for 24h. Leukocyte adhesion to liver sinusoidal endothelial cells in response to recombinant GREM1 was investigated using flow adhesion assays.

Results: Using RNAscope, we found increased expression of GREM1 mRNA in human and rat NASH fibrosis tissue with undetectable levels in healthy liver, while GREM1 was not consistently upregulated in murine liver fibrosis. In human NASH tissue, GREM1 localized to THY1⁺/Collagen 3A⁺ myofibroblasts in fibrotic septa. Additionally, GREM1 mRNA expression was increased 4.8 to 9.8-fold in human cirrhotic liver of different etiologies (Fig. A, $p = 0.015$; NASH, alcohol-related liver disease (ALD), PBC and PSC). Monoclonal aG1-Ab effectively inhibited BMP4-induced SMAD1 phosphorylation in LX2 cells *in vitro*, and showed target engagement *in vivo*, but was ineffective in reducing histological fibrosis and inflammation in a rat CDA-HFD model of NASH fibrosis (Fig. B). We determined the impact of aG1-Ab treatment in human disease by treating human cirrhotic PCLS with the aG1-Ab and saw no changes in fibrosis marker gene expression when compared to iso-Ab (Fig. C). Likewise on primary human myofibroblasts, aG1-Ab treatment was without effect on fibrosis marker gene expression. Recombinant GREM1 had no effect on leukocyte adhesion to liver endothelium in flow adhesion assays.

Conclusion: GREM1 expression is increased in human and rat NASH tissues. Despite evidence of target engagement, we were not able to identify any effects of GREM1 blockade on inflammation or fibrosis, suggesting redundancy of GREM1 signaling in the establishment and progression of NASH liver fibrosis.

Figure:



P01-13 Clinical translatability of the GAN diet-induced obese and biopsy-confirmed mouse model of NASH

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Background and aims: The Gubra-Amylin NASH (GAN) diet-induced obese (DIO) mouse is a highly validated preclinical model of non-alcoholic steatohepatitis (NASH). The present study aimed to compare hepatic transcriptome regulation during disease progression and back translation of drug treatment outcomes in the biopsy-confirmed GAN DIO-NASH mouse with reference to primary end point analysis in late-stage phase-II/III clinical trials in NASH patients.

Method: Liver biopsies were obtained from male C57Bl/6J mice fed the GAN diet (40 kcal-% fat, 22% fructose, 10% sucrose, 2% cholesterol) for 38-78 weeks. Global gene expression profiles were assessed by RNA sequencing and compared to published clinical data sets (Govaere et al. Sci Transl Med, 2020). Individual drug treatment studies were performed in GAN DIO-NASH mice with liver biopsy-confirmed NAFLD Activity Score (NAS \geq 5) and fibrosis stage (\geq F1). Mice were administered (QD) semaglutide (GLP-1 receptor agonist, 30 nmol/kg, SC), obeticholic acid (OCA) (FXR agonist, 30 mg/kg, PO), lanifibranor (pan-PPAR agonist 30 mg/kg, PO), resmetirom (THR- β agonist, 1 mg/kg, PO) or vehicle for 12 weeks. Histopathological pre-to-post individual assessment of NAS and fibrosis stage was performed and evaluated against primary end points (resolution of NASH with no worsening of liver fibrosis; \geq 1-stage fibrosis improvement without worsening of NASH), applied in corresponding clinical phase-II/phase III trials for semaglutide (Newsome et al. NJEM, 2020), resmetirom (Harrison et al. Lancet, 2019), lanifibranor (NATIVE: Francque et al. NJEM, 2021) and OCA (REGENERATE: Younossi, Ratziu et al. Lancet, 2019).

Results: GAN DIO-NASH mice reproduced regulations in clinical NASH core gene sets associated with fibrotic progression. Drug treatment efficacy on clinical histopathological end points in GAN DIO-NASH mice was comparable to corresponding clinical phase-2 trials with semaglutide, lanifibranor and resmetirom. In contrast, OCA treatment showed differential effects on clinical primary histopathological end points in GAN DIO-NASH mouse model and corresponding clinical phase-3 trial data in NASH patients.

Conclusion: Core NASH gene signatures of disease progression in GAN DIO-NASH mice are overall highly comparable to the human disease. Histopathological drug efficacy profiles in GAN DIO-NASH mice demonstrate back translation to compounds in advanced clinical development for NASH. This further validates the GAN DIO-NASH mouse model as a clinically relevant model of human NASH and highlights its utility in preclinical drug development.

P01-20-YI Antagonizing sodium taurocholate co-transporting polypeptide (NTCP) on NK cells from NALFD patients elevate their activity and mediate activated hepatic stellate cells killing

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Background and aims: 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 their activity potentials.

Method: Twenty-four patients biopsy-proven NALFD with liver fibrosis were classified according to Metavir scoring. 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 taurocholic acid (TCA, NTCP physiological substrate), Epigallocatechin 3-Gallate (EGCG) (NTCP antagonist) and HBsAg (NTCP agonist).

Results: NTCP were increased and linearly expressed on liver NK-cells of patient's biopsies and NK peripheral-blood of increasing severities of fibrosis scores. 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 on NK cells are involved in bile acid trafficking, TCA uptake into the cells was inhibited with an anti-NTCP neutralizing antibody and siRNA treatments, which resulted in NK cells re-activation and stimulated their potentials to kill activated HSCs in an in vitro setting. Moreover, 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.

Conclusion: Expressions of NTCP in NK-cells (known as anti-fibrotic cells) are associated with progression of liver fibrosis in NALFD patients and suggest their role in liver fibrosis. Modulatory therapies targeting BA-transporter NTCP, and NK cells could prevent complications to liver cirrhosis.

P01-21-YI Tissue resident NK NTCP- transplanted to immunosuppressed mice exhibiting liver fibrosis and fed with high fat diet (HFD) alleviate intestinal fibrosis and lipid profile

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Background and aims: NOD-*scid* *IL2ry*^{null} (NSG) mouse is one of the most widely used immunosuppressed mouse strains. We adapted this model as it exhibits absent T, B, and NK cells and allowed us to study the effects of transplanted NK cells expressing or not expressing the sodium taurocholate co-transporting polypeptide (NTCP); a transmembrane protein highly expressed in human hepatocytes that mediates the transport of bile acids.

Method: Tissue resident (tr) NK cells obtained from livers of naïve C.B-17 scid (having NK cells while lacking T and B cells) were sorted according to NTCP expressions and transplanted to the CCl₄-induced liver fibrosis immunosuppressed mice fed with HFD. Inflammatory (HandE staining, and pro-inflammatory panel of cytokines), fibrosis (Sirius red staining, α -smooth muscle actin, collagen, and fibronectin) and metabolic (BAs, cholesterol, triglyceride, glucose tolerance test (GTT) and fasting blood sugar (FBS)) profiles were assessed.

Results: Our data showing trNK^{NTCP+} displaying higher expressions of exhaustion markers of PD-1, TIGIT and LAG-3 as compared to their trNK^{NTCP-} counterparts. Moreover, these populations showed a reduction in their activation markers profile of NKp46, NKp30 and CD107a expressions. HandE staining from non-treated mice (NT) intestines showed swelled cells with and large necrotic areas of high infiltrating inflammatory cells with steatosis while mice transplanted with trNK^{NTCP+} showed a delayed in these histological findings with a significant reduction in micro- and macrovascular steatosis. Sirius Red staining in NT demonstrated increased collagen deposition in perisinusoidal areas; transplantation with trNK^{NTCP+} resulted in a remarkable reduction in the fibrous dense tissue of the stained area and significant inhibitions in α SMA and Col III in the trNK^{NTCP-} transplanted mice (1.8-fold and 3.1-fold, respectively; $p < 0.0002$) as compared to NT mice. These results were associated with reductions in pro-inflammatory (TNF- α , IL-1 β , IL-6 and IL-10) and pro-fibrotic (IL-4 and MCP-1) cytokines and amelioration in lipid profile of mice receiving the trNK^{NTCP+} while further significant reductions were obtained following the trNK^{NTCP-} transplantations ($p < 0.05$).

Conclusion: Our data clearly indicate effects of transplanted trNK^{NTCP-} in intestinal fibrosis amelioration and improving intestine histology of inflammation, fibrosis and lipid profiles indicating the involvement of bile acids in intestinal injury in obese mice.

P01-24 The role of calcium responsive NFATc1 and chronic ER stress signalling in NAFLD associated carcinogenesis

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Background and aims: NAFLD is a hepatic manifestation, arises by free fatty acids (FFA's) accumulation in hepatocytes, leading to inflammation, fibrosis and HCC. NFATc1 signaling has been reported to have fatal role in various inflammatory diseases and tumor development e.g., skin inflammation, PDAC etc. We investigate the functional role of calcium responsive NFATc1 in NAFLD progression to severe NASH and HCC, to develop therapeutic strategies.

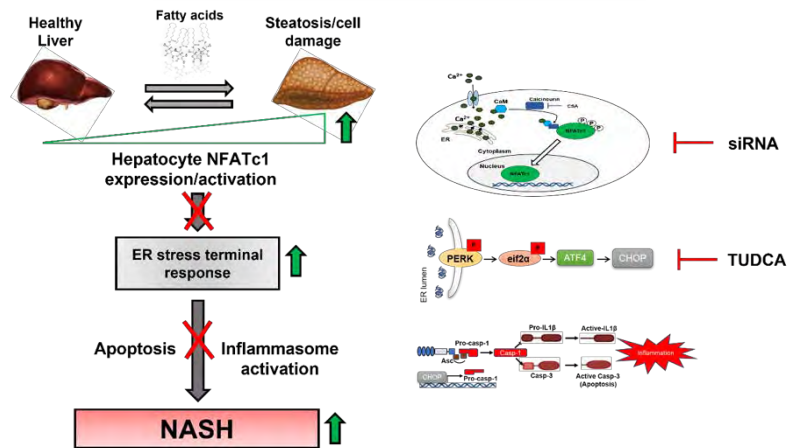
Method: NFATc1 activation was confirmed in NASH and HCC patients, cell lines and mice tissues pretreated with western-diet. Liver tissues from control-diet (CD) and western-diet (WD) fed NFATc1^{wt}, NFATc1^{c.a.} and NFATc1^{fl/fl} mice were examined for NFATc1 dependent morphological changes. NFATc1 regulated gene signatures and signaling mechanisms were identified by RNA-seq and therapeutic potential of NFATc1 dependent signaling mechanisms was examined by their inhibition.

Results: NFATc1 is highly activated in advanced human NAFLD/NASH and HCC. Moreover, WD-induced aberrant NFATc1 expression and activation promotes progressive hepatic inflammation and fibrosis in mice, whereas hepatocyte-specific depletion of the transcription factor can prevent from disease acceleration. Mechanistically, NFATc1 in response to altered calcium homeostasis, drives liver cell damage and inflammation through ER-stress sensing and activation of the PERK-CHOP unfolded protein response (UPR). Inhibition of ER-stress responses blocks NFATc1-induced disease progression towards NASH.

Conclusion: NFATc1 drives NAFLD progression through chronic ER-stress sensing and subsequent hepatocytes damage by inflammasome activation. Interfering with ER-stress responses, e.g., by TUDCA, protects fatty livers from progression towards NASH. These observations further suggest exploring the functional and therapeutic role of calcium signaling and NFATc1 in NASH related HCC development.

Figure:

NFATc1 drives NAFLD progression via regulating ER stress response signaling



P02-06 Hepatoprotective effects of semaglutide in the GAN diet-induced obese and biopsy-confirmed mouse model of NASH with advanced fibrosis and HCC progression

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Background and aims: The glucagon-like-receptor (GLP)-1 agonist semaglutide has demonstrated therapeutic efficacy on clinical end points in a recent phase 2 clinical trial in patients with non-alcoholic steatohepatitis (NASH) (Newsome et al. NEJM, 2021). The present study aimed to evaluate therapeutic efficacy of semaglutide on clinical end points and outcome in the GAN (Gubra-Amylin NASH) diet-induced obese (DIO) mouse model of NASH with advanced fibrosis and development of hepatocellular carcinoma (HCC).

Method: Male C57BL/6J mice were fed the GAN diet high in fat, fructose, and cholesterol for extended 48 weeks prior to study start. Only animals with liver biopsy-confirmed NAFLD Activity Score (NAS ≥ 5) and advanced fibrosis (stage F3) were included and stratified into treatment groups. DIO-NASH-HCC mice received (SC, QD) vehicle (n = 16) or semaglutide (30 nmol/kg, n = 15) for 14 weeks. Vehicle-dosed chow-fed C57BL/6J mice (n = 9) served as lean healthy controls. Untreated DIO-NASH-HCC mice (n = 10) were terminated at baseline. Tumor histopathological classification was performed by an expert clinical pathologist. Pre-to-post liver biopsy histopathology was performed for within-subject evaluation of NAFLD Activity Score (NAS) and fibrosis stage. Additional end points included blood biochemistry and quantitative liver histomorphometry.

Results: Compared to baseline, DIO-NASH-HCC mice demonstrated progressive HCC burden over the 14-week study period. Tumors showed consistent architectural and cytologic features of HCC with a marked loss of reticulin-stained fibers. Notably, semaglutide completely prevented progression in HCC burden. Concurrently, semaglutide improved hallmarks of NASH, including transaminases, hepatomegaly and histopathological NAS (≥ 2 point) without improving fibrosis stage. In agreement, semaglutide reduced quantitative histological markers of steatosis (lipids, hepatocytes with lipid droplets), inflammation (number of inflammatory foci, galectin-3), fibrogenesis (α -SMA), proliferation (Ki67) and progenitor cell activation (CK19).

Conclusion: This is the first study to demonstrate that semaglutide improves both clinical histopathological end points for NAFLD Activity Score and HCC burden in a preclinical translational mouse model of NASH-driven HCC. This highlights the suitability of GAN DIO-NASH-HCC mice for profiling novel drug therapies targeting NASH with advanced fibrosis and HCC.

P02-08 Therapeutic target identification by genome-wide RNAi screening in non-alcoholic fatty liver (NAFLD)

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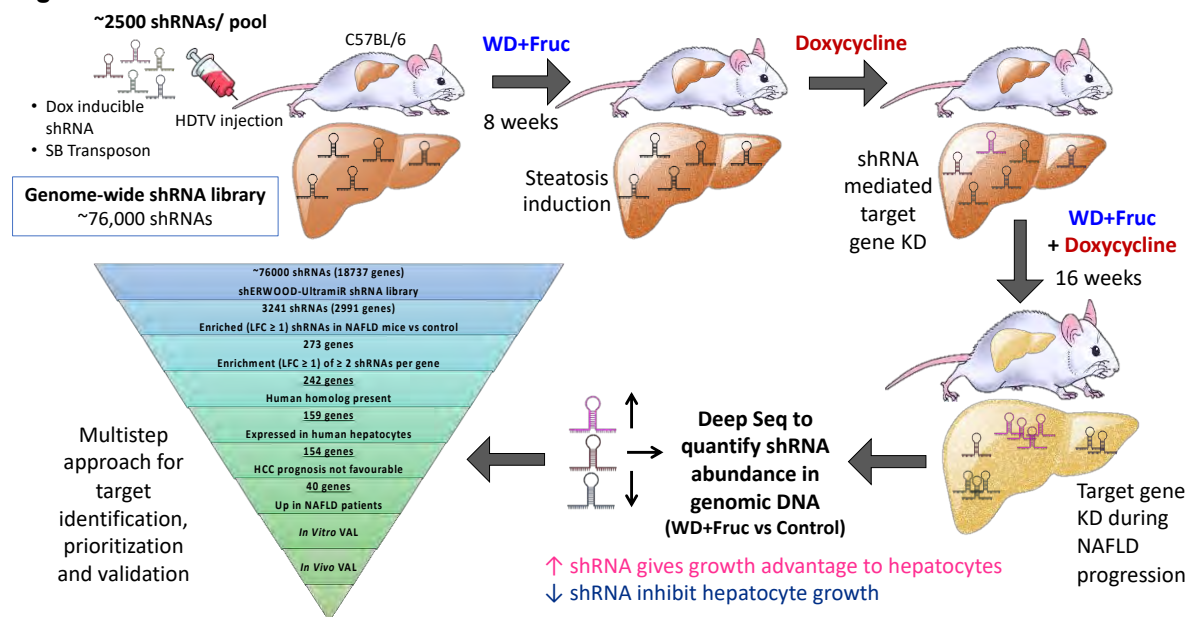
Background and aims: Chronic liver disease (CLD) is a global health burden. Non-alcoholic fatty liver disease (NAFLD) is fast becoming the leading cause of CLD due to rise in the incidence of obesity and metabolic disorders. However, the only treatment option available to CLD patients is liver transplantation and there are no clinically approved drugs for NAFLD. Therapies that can reverse the progression of NAFLD and/or regenerate the damaged liver tissue are urgently needed. Here we conducted a genome-wide RNAi screen in NAFLD mouse model to identify novel targets with potential for liver regeneration.

Method: A genome-wide mouse shRNA library (shERWOOD-UltramiR) was cloned into transposon-based doxycycline (Dox)-inducible vector, divided into 32 pools and delivered into C57BL/6 mice liver using hydrodynamic-tail vein injections. Mice were fed Western diet and fructose-supplemented water (WD+Fruc) for 8 weeks followed by induction of shRNA expression by Dox administration and continued diet feeding for additional 16 weeks. Liver tissues were harvested followed by deep sequencing and differential expression analysis using multiple bioinformatics tools to identify shRNAs that are enriched or depleted in NAFLD mice (WD+Fruc fed) as compared to control (chow fed) mice. Top candidate genes were selected based on a number of stringent criteria and validated using *in vitro* and *in vivo* liver regeneration models.

Results: Deep sequencing and differential expression analysis identified ~500 genes with shRNAs enriched or depleted in the NAFLD mouse liver. Pathway analysis confirmed the enrichment of genes involved in lipid biosynthetic process and extracellular matrix organization. We identified 273 genes with multiple shRNAs enriched in the NAFLD mouse liver. This gene list was curated to further select only those genes that are expressed in the human hepatocytes and whose inhibition is not expected to promote tumour growth (154 genes). Target genes with upregulated expression in the NAFLD patient transcriptome were prioritised for further validation. *In vitro* analysis of the selected candidates in mouse hepatocyte cell lines showed enhanced cell migration and proliferation ability upon shRNA mediated gene knockdown (KD). Further evaluation of successful candidates *in vivo* using clonal cell expansion assay in fumarylacetoacetate hydrolase (*FAH*^{-/-}) transgenic mice confirmed higher liver regeneration potential of the top target gene shRNAs. Ongoing studies in the *FAH*^{-/-} mice under WD+Fruc diet feeding will validate the impact of target gene KD on NAFLD histopathologies including fibrosis and steatosis.

Conclusion: An unbiased multistep *in vivo* RNAi screen identified potential novel therapeutic targets for NAFLD. The inhibition of these targets leads to increase in the regenerative capacity of the liver which can counteract liver damage and prevent NAFLD disease progression.

Figure:



P02-09 Identifying novel epigenetic regulators of non-alcoholic fatty liver disease using *in vivo* RNAi screen

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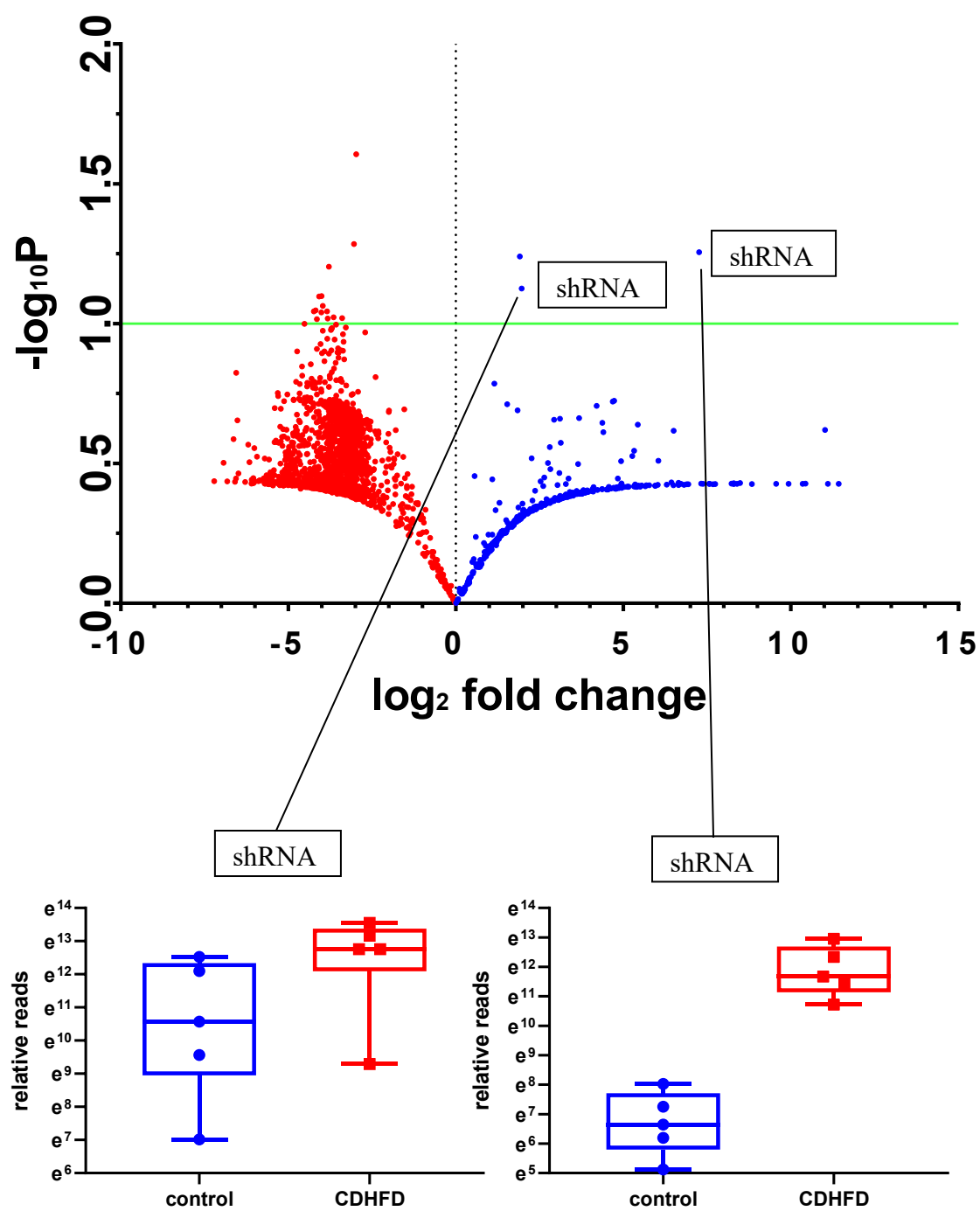
Background and aims: With a rapidly increasing prevalence, non-alcoholic fatty liver disease (NAFLD) is recognized as the most common cause of chronic liver disease worldwide. The massive growth of NAFLD is causing a significant burden in health care with social and economic implications. However, unlike other highly prevalent diseases, it remains an underrepresented disease with limited therapeutic possibilities due to the absence of current approved pharmacological treatments. Therefore, there is an urgent need to develop innovative therapies. To address this, we are utilizing an *in vivo* functional genetic screen with disease mouse models.

Method: We used functional genetic approaches to determine the impact of epigenetic modulators on disease manifestation and progression. We performed unbiased *in vivo* functional genetic screens for epigenetic modifiers. The library was directly and stably delivered into mouse hepatocytes hydrodynamic injection. Mice were fed with Choline deficient L-amino acid defined high-fat diet (CDHFD) to recapitulate human pathology of the NAFLD. As a control, normal chow-fed animals were served. Our approach's underlying idea is that shRNAs that confer benefits to the hepatocyte in the detrimental environment of the disease will be enriched over time. At the end of diet treatment, livers were harvested, and the abundance of each shRNA was determined by deep sequencing. Highly enriched shRNAs were validated for their potential therapeutic impact by various studies, including *in vitro* assays for proliferation and *in vivo* by repopulating FAH^{-/-} mice so that every hepatocyte expresses the shRNA of interest. Repopulated mice were exposed to CDHFD diets, and histopathology parameters were evaluated.

Results: Our screens have identified novel shRNAs highly enriched in the CDHFD model. *In vitro* knockdown of target genes showed significant increase in cell proliferation and migration rate. Selected shRNAs has been validated for reducing liver fibrosis in the CDHFD mouse model.

Conclusion: We showed that the *in vivo* functional genetic screening in the disease mice model could discover new candidates as therapeutic targets of NAFLD.

Figure: RNAi screen under CDHFD, significant enriched shRNAs selected for validation



P02-11-YI Yeast β -glucan improves insulin sensitivity and hepatic lipid metabolism in mice humanized with obese type 2 diabetic gut microbiota

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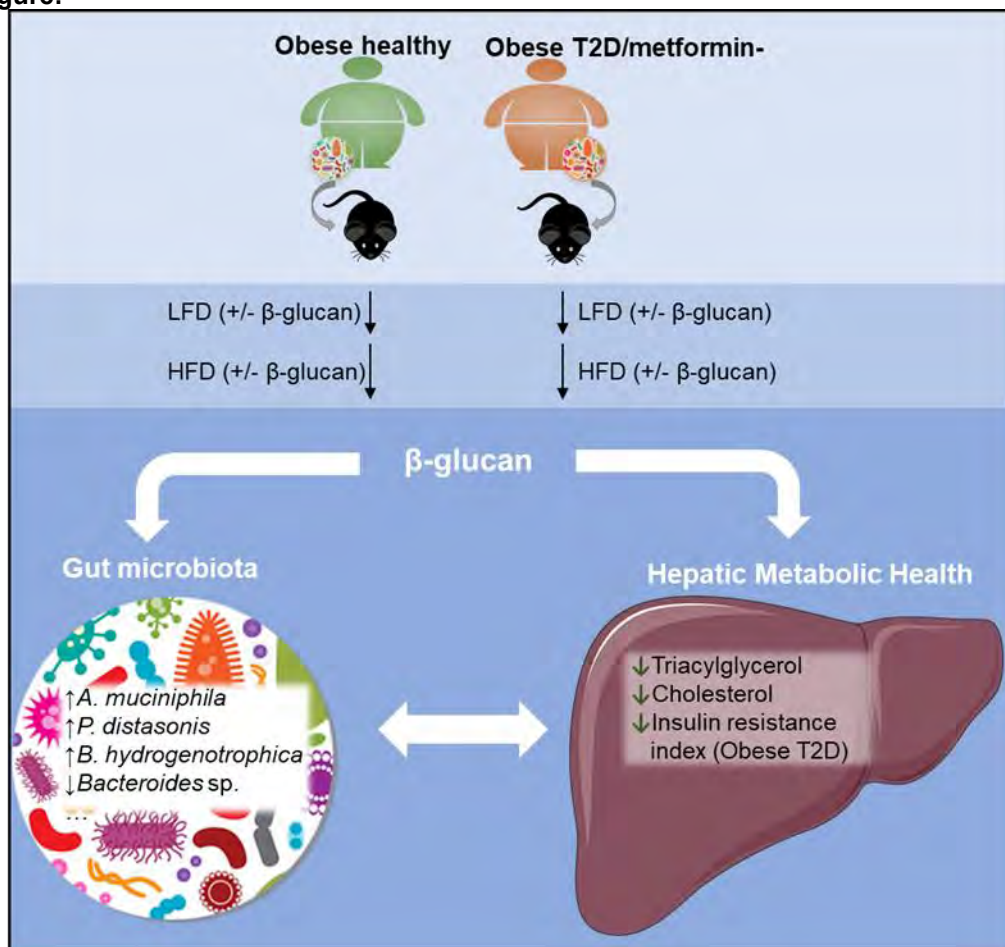
Background and aims: Gut microbiota composition is impacted by obesity, type 2 diabetes (T2D) and dietary challenges. T2D is an inflammatory condition which promotes glucose intolerance and insulin insensitivity. β -glucans are naturally occurring diverse group of polysaccharides which can improve lipid homeostasis, cholesterol metabolism, glucose levels and microbiome composition. This study focused on how yeast-beta-glucan may interact with the human microbiome with respect to metabolic and liver health. Our aim was to determine whether baker's yeast (1 \rightarrow 3)- β -D-glucan (β G) interacts differently with a human obese healthy versus obese diabetic gut microbiome, to effect the hepatic proteome and metabolic health, in response to a high-fat diet.

Method: C57BL/6J male mice received an antibiotic cocktail in the drinking water to diminish the endogenous microbiota population for 6 weeks. Mice were then orally gavaged with microbiota samples obtained from obese healthy (OBH) or diabetic (OBD) humans twice daily for 3 days. Mice were fed a low fat diet (LFD) (10% kcal) for 4 weeks followed by HFD (45% kcal) for 9 weeks. Half of OBH and OBD mice received diets enriched with β G (OBH \pm β G and OBD \pm β G). Following HFD challenge, glucose tolerance (1.5g/kg), insulin tolerance (0.5U/kg) and gut microbial compositions were assessed. Liver was harvested and examined for triacylglycerol (TAG) formation, cholesterol accumulation, lactate and citrate levels. Metabolic markers were measured by real-time RT-PCR. Hepatic mass-spectrometry quantitative proteomics was completed to determine the altered metabolic pathways to compliment the phenotypic data.

Results: OBD mice were more glucose intolerant and insulin insensitive than the OBH counterparts. Additionally, fasting HOMA-IR, attributable to higher insulin concentrations, was higher in OBD mice. β -glucan supplementation reduced HOMA-IR in OBD mice. Despite equal total weight gain, microbiome source had a significant effect on hepatic metabolic health and inflammation. OBD mice had higher TAG and cholesterol levels which trended to be reduced by β G. Livers from OBD mice also displayed higher lactate and citrate levels. Hepatic proteomics demonstrated that OBD microbiome transplantation increased HFD-induced hepatic mitochondrial dysfunction, disrupted oxidative phosphorylation, and reduced protein synthesis, which were partly reverted by yeast β -glucan supplementation

Conclusion: Different human microbiome transfer acted as differential modulators of insulin resistance and hepatic health, despite equivalent obesity. Interestingly dietary supplementation with yeast β -glucan altered the microbiome and improved metabolic and hepatic health. Nevertheless the causal versus coincidental role of yeast β -glucan on the microbiome in mediating metabolic health requires further investigation.

Figure:



P02-18-YI The paraoxonase gene family in the etiology and progression of obesity and associated liver disease: a genetic case-control study

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Background and aims: Obesity is accompanied with excessive oxidative stress and inflammation, processes that are mainly known to play a paramount role in the progression of obesity-associated comorbidities such as NAFLD. Interestingly however, free radicals (inducers of oxidative stress) are also capable of inducing liver steatosis independently in mice by mechanisms similar to those induced by a high-fat diet. Steering away from the classic “two-hit” hypothesis and embracing the “multiple hit” hypothesis, we focused on a family of paraoxonase (*PON*) genes that has anti-inflammatory and anti-oxidative properties and is a potential candidate in the etiology of both obesity and obesity-associated liver disease.

Method: The possible role of *PON1*, *PON2* and *PON3* in obesity and obesity-associated liver disease was examined by assessing enrichment of rare variants in these genes using variant burden and variance component testing. To this end, 507 lean controls (BMI 18.5-25) and 793 obese (BMI >30) individuals with (out) associated liver disease were sequenced for *PON1*, *PON2* and *PON3* using the single molecule Molecular Inversion Probes (smMIPs) technology.

Results: Under a wide range of models, *PON1* is significant for rare (minor allele frequency (MAF) <0.01) and very rare (MAF <0.0012) variants when comparing controls with cases with the sole exception of the variance component test for very rare variants. As liver biopsies of all individuals with NAFLD were meticulously characterized, we were also able to test for associations with several NAFLD-associated liver injury parameters including steatosis, inflammation, fibrosis and ballooning grade. Very significant results were found for *PON2* under a wide range of models in both rare and very rare variants when comparing samples without liver fibrosis to samples with varying degrees of liver fibrosis.

Conclusion: These results indicate a relevance for *PON1* in the etiology of obesity. Moreover, to our knowledge, we are the first to report that *PON2* might be a key player in the etiology and/or the progression of liver fibrosis.

Figure:

Case- control: *PON1*

Test	Rare variants	Very rare variants
<i>c-alpha</i>	0.0084	0.3716
<i>Cfisher</i>	0.0028	0.0429
<i>Kbac</i>	0.0004	0.0072
<i>vt</i>	0.0036	0.0388

No fibrosis vs varying stages of fibrosis: *PON2*

Test	Rare variants	Vary rare variants
<i>c-alpha</i>	0.001	0.002
<i>Cfisher</i>	0.003	0.006
<i>Kbac</i>	0.003	0.006
<i>vt</i>	0.001	0.003

Table 1. p values for significant variant burden (*Cfisher*, *Kbac*, *vt*) and variance component (*c-alpha*) tests in cases versus controls for *PON1* and no fibrosis versus varying stages of fibrosis for *PON2*.

P02-21-YI Investigation of the plasma proteome to identify drug targets for non-alcoholic fatty liver disease and metabolic dysfunction-associated fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a leading cause of liver disease with slow progression and absence of pharmacological treatment. Metabolic dysfunction-associated fatty liver disease (MAFLD) is a new trait definition that does not include alcohol consumption. We aimed to study the joined molecular mechanism of NAFLD and MAFLD at the genetic and proteomic level and explore potential drug targets.

Method: We analysed 1, 472 plasma proteins measured using the Olink platform in 44, 031 randomly selected European participants from UK Biobank, including 729 patients with NAFLD and 16, 927 with MAFLD. Logistic regression was performed for associations of proteins and NAFLD or MAFLD. We investigated the genetic and proteomic correlation between NAFLD and MAFLD using LD-regression and protein associations. We further used multiple genetic methods, such as genome-wide association studies (GWAS) and Mendelian randomization (MR), to identify proteins that may be potential drug targets of NAFLD or MAFLD.

Results: We identified 1, 145 proteins cross-sectionally associated with NAFLD or MAFLD. We found a high genetic correlation ($r_g = 0.84$) between NAFLD and MAFLD_{excl.NAFLD}, and 98.5% of the NAFLD-associated proteins were also associated with MAFLD_{excl.NAFLD}. The proteomic correlation between NAFLD and MAFLD_{excl.NAFLD} is 0.93. We performed GWAS for each of the proteins and identified 16, 034 cis-protein quantitative trait locus (cis-pQTLs). Using MR, we identified three proteins associated with NAFLD and 13 with MAFLD ($p < 3.3 \times 10^{-4}$). Among them, GGT1 was highlighted by multiple methods, including MR ($p = 3.4 \times 10^{-43}$), colocalization (Posterior Probability = 0.99), and rare variant analysis (22:24627868:T:C, $p = 2.4 \times 10^{-5}$). It is also associated with non-alcoholic steatohepatitis (beta = 1.7, $P = 1.2 \times 10^{-22}$). We did not find any adverse effects of perturbing GGT1 using phenome-wide association studies.

Conclusion: Our study shows that genetic and proteomic signatures between NAFLD and MAFLD strongly overlap, suggesting that MAFLD may be a common trait that allows powerful aetiological and intervention studies. We identify 15 potential drug targets of NAFLD or MAFLD. GGT1 is one of the consistent drug proteins emerging for fatty liver disease.

P03-04 Estrogen-related receptor alpha regulates ribosomal stalk protein RPLP1-dependent translation of lysosome and autophagy proteins in fasting and non-alcoholic steatohepatitis (NASH).

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Background and aims: Translation regulation of lysosome and autolysosome proteins in fasting and non-alcoholic steatohepatitis (NASH) is not well studied.

Method: We used unbiased proteomic analysis in hepatic cells, gene manipulation in vitro and in vivo, and dietary mouse models of starvation and NASH in this study.

Results: Global protein translation during acute fasting was decreased. However, an unbiased proteomic analysis revealed specific subsets of proteins corresponding to ribosomes and lysosomes were translated during prolonged fasting. Further analysis showed that Esrra was required for the transcriptional induction of Rplp1 and the translation of lysosomal/autolysosome proteins that sustained lysosome-autophagy function in cell culture and mouse models during starvation. Interestingly, hepatic Esrra-Rplp1-dependent translation of lysosomal proteins and autophagy also was impaired in patients and mice with non-alcoholic steatohepatitis (NASH). Remarkably, genetic activation of Esrra or alternate-day fasting increased Esrra expression, induced Rplp1 expression, restored general protein translation, increased expression of lysosomal proteins, induced autophagy, and reduced lipotoxicity, inflammation, and fibrosis in cell culture and in in vivo models of NASH.

Conclusion: Esrra regulated ribosomal-dependent translation of lysosome/autolysosome proteins during prolonged fasting/starvation. This pathway also was dysregulated in NASH; thus, Esrra and Rplp1 may serve as therapeutic targets to activate this pathway during NASH. We also showed for the first time that a nuclear hormone receptor, Esrra, not only regulated transcription but also protein translation through its induction of Rplp1.

P03-09 Oxygen-nutrient mismatch correction by obeticholic acid suggests mechanism of action in NASH

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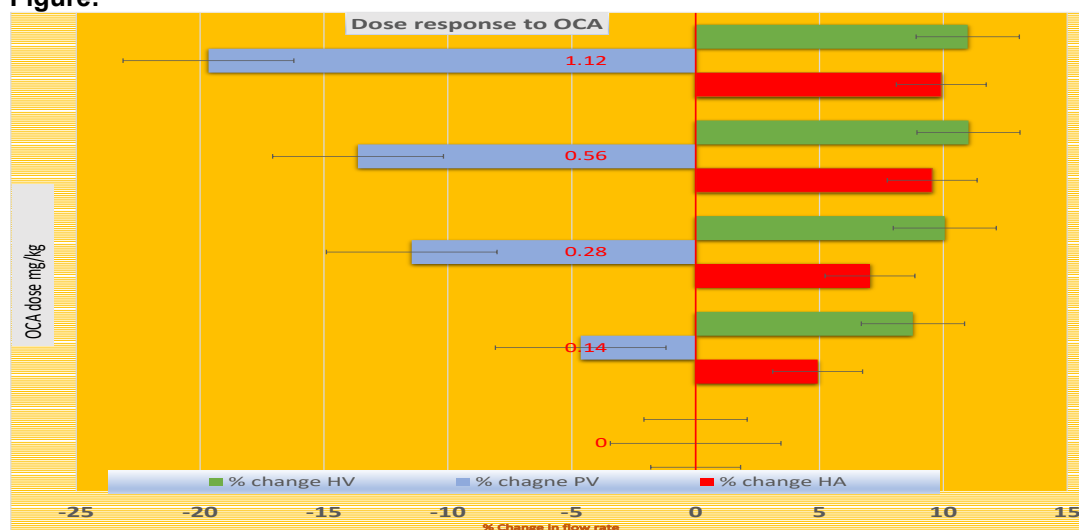
Background and aims: Accumulating evidence linking intermittent hypoxemia and NAFLD, prompted us to revisit the concept of Oxygen-Nutrient mismatch as potential target for the therapeutic efficacy of Obeticholic acid (OCA) in NASH. The liver has a unique dual blood supply. The hepatic artery (HA) provides 20 percent of blood flow but delivers 50 percent of oxygen supply. Portal vein (PV) blood is deoxygenated and highly enriched with nutrients. PV flow lacks myogenic regulation. This raises the potential for oxygen-nutrient mismatch and relative tissue hypoxic damage to the liver, specifically in NASH. The aim of this study was to determine whether the effect of increasing doses of obeticholic acid on flow rates in the HA, PV, and hepatic vein (HV) using fresh porcine livers, perfused *ex vivo* with a cardio-emulation pump CaVESWave®, under constant pressure conditions, might provide an explanation for the benefit of OCA in NASH.

Method: Liver perfusion was initiated with the HA settings at 120/80 mmHg, PV below 15 mmHg and temperature at 15° C. Sensing data collected included flow rates on all three vascular channels. The treatment protocol commenced after the hepatic venous outflow stabilized beginning with the initial dose of OCA, 0.14 mg/kg weight of liver and increasing doses of 0.28, 0.56 and 1.12 mg/kg, added at 30 min intervals.

Results: Hepatic artery pressure measurements varied within a narrow range of systolic (117-122 mmHg) and diastolic (75-84 mmHg) pressures (fig 1). Perfusate pH varied narrowly between 7.35-7.4; DO between 104-105; and temp 15.8-17.15° C. The control livers (n = 2) showed only minor changes in flow after treatments with vehicle. The maximum percentage increase in HA flow was 3.3 ± 3.5 and 4.8 ± 2.8 for the HV. PV flow fell by -9.1 ± 5.9 . In the drug treated livers (n = 4), there was a clear dose-responsive relationship between OCA and HA, HV and PV. HA flow increased in dose-response fashion by 9.9 ± 8.9 percent. Hepatic venous outflow increased progressively by 11 ± 11.8 percent. By contrast, PV flow fell by -19 ± 16 percent (fig.1).

Conclusion: OCA in dose responsive manner increased HA flow and simultaneously reduced PV flow. This inverse relationship between HA and PV flow, likely reflects hepatic artery buffering response. Increasing oxygenated HA blood flow and simultaneously reducing nutrient rich PV flow serves to correct oxygen-nutrient mismatch and could account for the beneficial effect of OCA in NASH progression.

Figure:



P03-13 The SEMA7A_R148W mutation promotes lipid accumulation and NAFLD progression by increasing its localization on hepatocyte surface

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Background and aims: Genetic polymorphisms are associated with the development of non-alcoholic fatty liver disease (NAFLD). *Semaphorin7a* (*Sema7a*) deficiency in mouse peritoneal macrophages reduces fatty acid (FA) oxidation.

Method: To examine whether SEMA7A mutations contribute to the development of NAFLD in humans, we performed the exon sequencing of *SEMA7A* in 470 biopsy-proven NAFLD patients. We generated *Sema7a*^{R145W} heterozygous mice (equivalent to human *SEMA7A*^{R148W}) to further investigate the functional role of *SEMA7A* mutations in the progression of NAFLD.

Results: We identified 17 individuals with *SEMA7A* heterozygous mutations in 470 biopsy-proven NAFLD patients. *SEMA7A* heterozygous mutations increased susceptibility to NAFLD (OR = 12.75; 95%CI: 7.33-22.16), steatosis severity and NAFLD activity scores (NAS) in humans and mice. The *Sema7a*^{R145W} mutation significantly induced small lipid droplet accumulation in mouse livers. Mechanistically, the *Sema7a*^{R145W} mutation increased N-glycosylated Sema7a and its receptor integrin $\beta 1$ proteins in hepatocyte-surface membranes, and subsequently caused intrahepatic lipid droplet accumulation by enhancing the PKC α -stimulated FA and triacylglycerol (TG) synthesis and FA uptake.

Conclusion: *SEMA7A*^{R148W} mutation is a new strong genetic determinant of NAFLD, and promotes intrahepatic lipid accumulation and NAFLD in mice through enhancing the PKC α activation. Inhibiting the activation of hepatic PKC α signaling may be a novel therapy for NAFLD.

Figure:

Figure 1. The *Sema7a*^{R145W} heterozygous mutation promotes the progression of NAFLD in mice following HFD feeding. Figure 2. The *Sema7a*^{R145W} mutation does not alter total Sema7a expression, but increases Sema7a and its receptor integrin $\beta 1$ proteins in cell-surface membrane and activates the PKC α signaling in hepatocytes.

P03-14-YI Branched-chain amino acid catabolism and hepatic stellate cell activation

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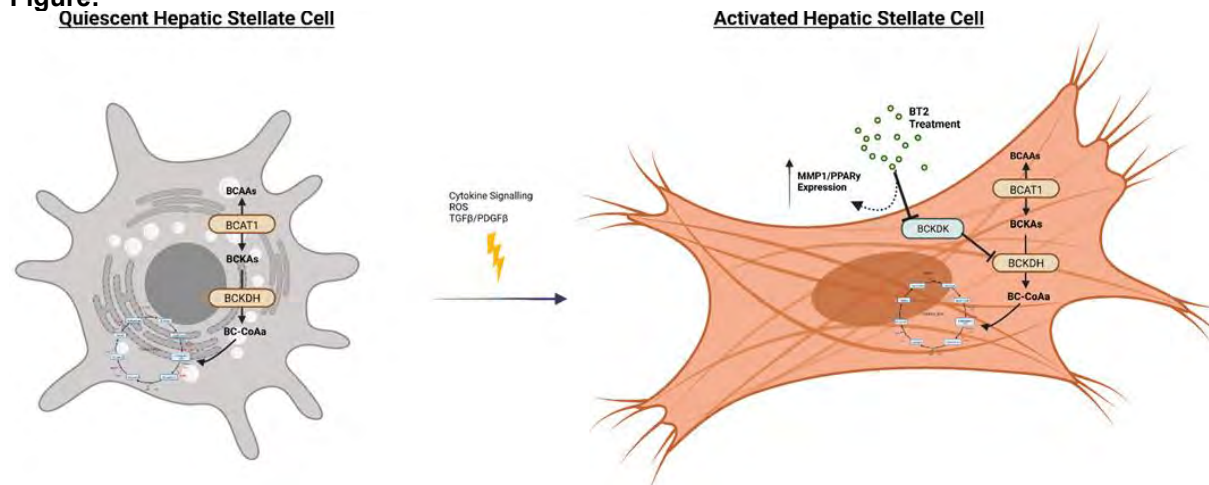
Background and aims: NAFLD/NASH is a progressive and heterogeneous disorder with fibrosis score being a key indicator of patient outcome severity. In both healthy and diseased liver the hepatic stellate (HSC) is the primary cell responsible for extracellular matrix (ECM) deposition and remodelling. During NASH, HSCs become transactivated into proliferative, migratory, and contractile myofibroblast like cells without means of resolution. Targeting this transformation has become an area of interest for treatment of more severe NAFLD/NASH cases. The role of metabolism is common to many cell transformations due to the high energy demand associated with an increase in proliferation, migration, anabolism, and the restructuring of the extracellular environment. Targeting metabolism may prove to be a promising avenue for inhibiting or resolving HSC activation. Branched-chain amino-acid (BCAA) catabolism has been identified as an important metabolic pathway with influence in various cell transformations such as adipocyte differentiation, pancreatic stellate cells, and proximal tubule cells during kidney fibrosis. The aim of this study is to investigate if BCAA catabolism is altered during HSC activation.

Method: A meta-analysis was conducted of publicly available RNAseq datasets (GSE78853, GSE119606, GSE151251, and GSE127964) examining the metabolic gene signature of TGF β induced activation of primary, human hepatic stellate cells (HSCs). *In vitro* experiments were conducted on the immortalized hTERT-HSC and LX-2 cell lines using TGF β as a model of activation. BT2 was used to target the branched-chain amino acid catabolic pathway. RT-PCR was used to monitor expression of key markers of HSC activation/quiescence. Metabolic activity of specific pathways was analysed using stable isotope tracing and GC-MS analysis of TGF β treated cells.

Results: Results of transcriptomic meta-analysis identified the BCAA catabolic pathway as being downregulated during HSC activation. Preliminary investigation of HSC cell lines treated with the BCKDK inhibitor, BT2, to increase BCAA catabolism showed an increase in expression of the quiescence markers PPAR γ and MMP-1. Cells treated with TGF β also displayed a decrease in expression of the rate limiting enzyme of BCAA catabolism, BCKDH (see figure below). Finally, analysis of metabolite uptake fluxes indicated a decrease in BCAA uptake with TGF β treatment.

Conclusion: Preliminary results from investigation of the effect of BT2 treatment on immortalized HSCs *in vitro* suggest that BCKDH is put under active inhibition by BCKDK during activation and that alleviating this inhibition can increase expression of key expression markers of HSC quiescence (see figure below).

Figure:



P03-21-YI Beta 7 integrin-mediated intestinal migration of pro-inflammatory monocytes contributes to western-style diet-induced obesity and non-alcoholic fatty liver disease in mice

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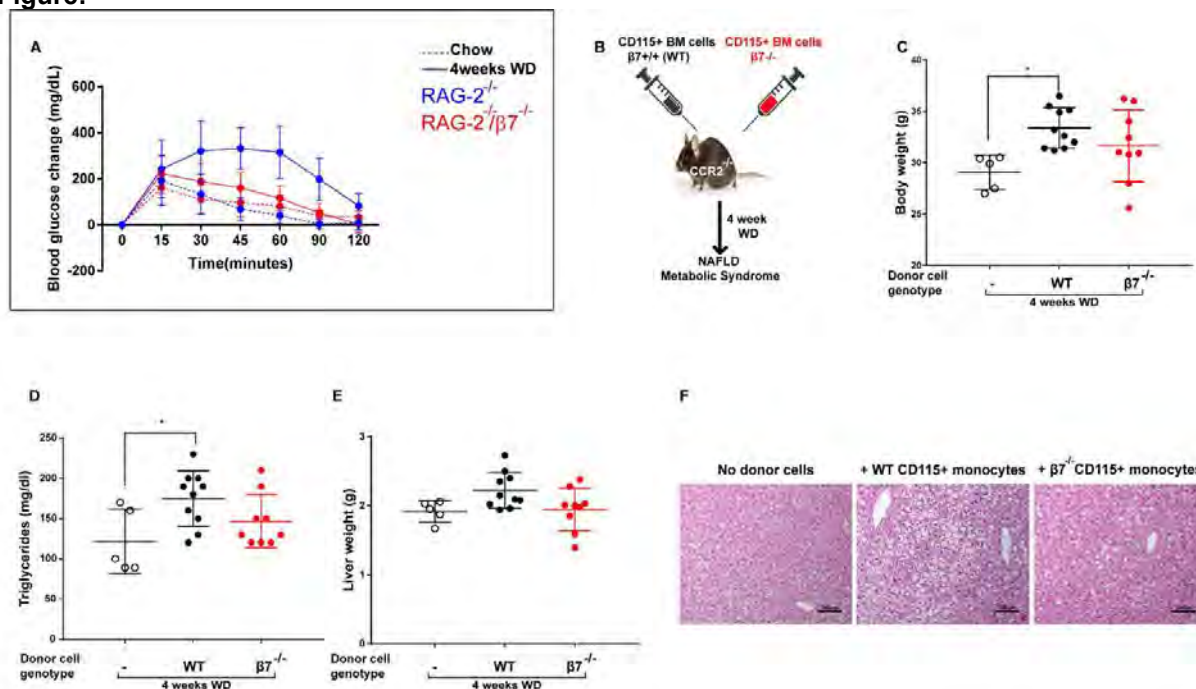
Background and aims: Western-style diet (WD)-induced obesity is a key risk factor for the development of non-alcoholic fatty liver disease (NAFLD) and is associated with a state of chronic low-grade inflammation. NAFLD is the hepatic manifestation of metabolic syndrome. Monocyte-derived macrophages contribute to the inflammatory processes underlying metabolic dysfunction and NAFLD. In response to chemokine and cytokine signaling, bone marrow derived monocytes differentiate into diverse subsets of pro-and anti-inflammatory monocytes, which migrate to adipose tissue, intestine, and liver. C-C chemokine receptor-2 (CCR-2) regulates monocyte recruitment in NAFLD while CCR2 inhibition reportedly improves metabolic dysregulation and inflammation in mice. During homeostasis, constitutive homing of monocytes to the gut is jointly mediated by CCR2 and beta 7 integrin. This study evaluates the role of beta7 integrin for monocyte recruitment in diet-induced metabolic dysregulation and NAFLD in mice.

Method: Lymphopenic recombination activation gene (RAG)-2-deficient and beta7 integrin/RAG-2 double-deficient mice were compared in a 4-week model of WD containing high-fat, fructose, and cholesterol. CD115⁺ BM monocytes from wild-type (WT) or beta7 integrin-deficient mice were compared by adoptive transfer into CCR-2 deficient mice which were subsequently subjected to 4 weeks of WD feeding. Diet-induced changes in gut, liver and epididymal white adipose tissue (EWAT) were measured by histology, serology, flow cytometry, ELISA, and RT-PCR.

Results: WD-fed RAG-2-deficient mice showed a worsened glucose tolerance, in comparison to similarly-fed beta7 integrin/RAG-2 double-deficient mice. Improved glucose tolerance was accompanied by significantly reduced numbers of Ly6C⁺ expressing newly extravasated monocytes in the colonic lamina propria of beta7 integrin/RAG-2 double-deficient mice but did not significantly hinder NAFLD progression. Transfer of CD115⁺ WT BM monocytes into CCR2-deficient recipients, subsequently placed on a WD diet, significantly aggravated metabolic parameters leading to increased body and liver weight, and higher levels of circulating triglyceride in comparison to the respective non-transferred mice. In addition, it aggravated the characteristic histological signs of NAFLD in the liver with increased steatosis and hepatocyte ballooning. This effect was less pronounced after transfer of beta7 integrin-deficient monocytes and was accompanied by a reduced migration of beta7 integrin-deficient monocytes into the colonic lamina propria.

Conclusion: Beta7 Integrin contributes to the development of immune-mediated inflammation in diet-induced metabolic dysregulation and to NAFLD progression by directing inflammatory monocytes into the intestine.

Figure:



β7 integrin-expressing monocytes contribute to WD-induced metabolic dysregulation and NAFLD development. (A) Intrapерitoneal glucose tolerance test (IPGTT) in chow-fed RAG-2^{-/-} (n= 5) and RAG-2^{-/-}/β7^{-/-} (n= 13) and WD-fed RAG-2^{-/-} (n= 8) and RAG-2^{-/-}/β7^{-/-} (n= 10) mice. (B) Experimental design for (C-F) adoptive transfer of Wild Type or β7 integrin-deficient-CD115 monocytes into CCR2-deficient mice. (C) Endpoint body weight. *p value < 0,05 (D) Serum triglyceride levels. *p value < 0,05 (E) Liver weight (F) Representative H&E stained images showing histological features of NAFLD. Scale bar=100μm

P03-23 Obeticholic acid modulates DDAH-ADMA-eNOS axis and attenuates portal pressure in an experimental non-alcoholic fatty liver disease

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Background and aims: Endothelial dysfunction (ED) is an early and relentless event in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). Improving endothelial function may be a promising approach to preventing NAFLD progression and associated complications. Here, we aimed to elucidate the role of obeticholic acid (OCA) in regulating the hepatic DDAH-ADMA-eNOS axis and associated portal pressure reduction in mice with fatty liver disease.

Method: Male CD-1 mice (Swiss strain) were fed a high-fat diet (HFD; Dyets, Inc. PA, USA) daily for three months to induce fatty liver. Moreover, NAFLD mice were orally administered OCA-INT-747 (Sigma, USA; 5mg/kg b.w. daily by gastric lavage) in the vehicle (corn oil) for 14 days or vehicle alone. At the end of 14 weeks, all mice in different study groups were sacrificed. Blood and liver tissue were harvested for various analysis.

Results: We found significantly decreased hepatic phosphorylated endothelial nitric oxide synthase (peNOS) and dimethylarginine diamino hydrolase (DDAH) 1 expressions whilst increased DDAH 2 in HFD mice. OCA-INT-747 treatment to NAFLD mice shows significantly increased peNOS and DDAH - 1 expressions. Moreover, hepatic and blood ADMA concentrations were significantly increased in NAFLD mice and were decreased following treatment with OCA in NAFLD mice. Importantly, OCA treatment reduces portal pressure and associated endothelial dysfunction in NAFLD mice. In addition, inflammatory markers such as TNF- α , IL1 β , NF κ b and iNOS were insignificantly elevated and 4HNE was significantly increased in NAFLD and were downregulated following OCA treatment to NAFLD mice.

Conclusion: Our novel data suggest that OCA-INT-747 treatment to NAFLD mice could modulate the hepatic DDAH-ADMA-eNOS axis and ameliorates portal pressure and thus ED. Targeting DDAH-1-based gene therapy may reduce ED and be a future therapeutic approach to managing NAFLD patients.

P04-04-YI Intercellular adhesion molecule-1 is involved in western diet-induced liver damage and metabolic regulation in mice

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Background and aims: Obesity is a key risk factor for the development of non-alcoholic fatty liver disease (NAFLD). NAFLD is associated with metabolic dysfunction, low grade inflammation of adipose tissue, dysbiosis, and enhanced gut barrier permeability. We still have no detailed understanding of the mechanistic links, which is a prerequisite for the development of therapies against NAFLD progression. Immune cell migration is known to link inflammatory processes in different organs and is guided by chemokines and adhesion molecules. In this study, we examined the role of intercellular adhesion molecule-1 (ICAM-1) in metabolic regulation and obesity-mediated liver disease in mice.

Method: ICAM-1-deficient mice and wild-type (WT) mice were compared in a 24-week Western-style diet (WD) model. The degrees of NAFLD, metabolic alterations, gut-barrier integrity, and microbial diversity were evaluated by histology, serology, flow cytometry, real-time PCR (RT-PCR), colorimetric assays, and 16S rRNA gene amplicon sequencing.

Results: ICAM-1 expression was induced in the liver and gut of WT mice by WD feeding. No differences in food intake, weight gain, liver triglyceride levels, or liver free fatty acids levels were found in mice of either genotype. We also detected no difference in the degree of fibrosis as exemplified by sirius red staining or collagen 1 alpha expression of liver tissue. However, WD-fed ICAM-1-deficient mice exhibited a significantly lower NAFLD activity score and decreased serum levels of liver transaminases, when compared to similarly fed WT mice. Flow cytometric analysis revealed increased numbers of neutrophils in livers of WD-treated ICAM-1-deficient mice, but not in WT livers. A stronger infiltration of immune cells into adipose tissue was visible in WD-fed ICAM-1 deficient mice. This finding was supported by flow cytometric analysis which revealed increased numbers of CD11b⁺F480⁺ macrophages and Ly6G⁺ neutrophils. Moreover, WD-fed ICAM-1-deficient mice exhibited more pronounced features of the metabolic syndrome when compared to similarly treated WT mice, as demonstrated by a reduced glucose tolerance. Irrespective of the genotype, WD feeding caused a shortening of the colon in comparison to chow-fed controls, but no obvious damage was evident on histological scoring of the colon and large intestine. Interestingly, ICAM-1-deficient mice showed a significantly higher intestinal expression of the tight junction proteins Zonula occludens-1 and Claudin-5, compared to WT mice, suggesting an improved gut barrier function. Changes in fecal microbiota profiles with decreased microbial diversity were observed in both mouse strains following WD feeding.

Conclusion: ICAM-1 appears to promote diet-induced liver damage in mice and affects gut barrier integrity. On the other hand, it protects from metabolic dysregulation and adipose tissue inflammation.

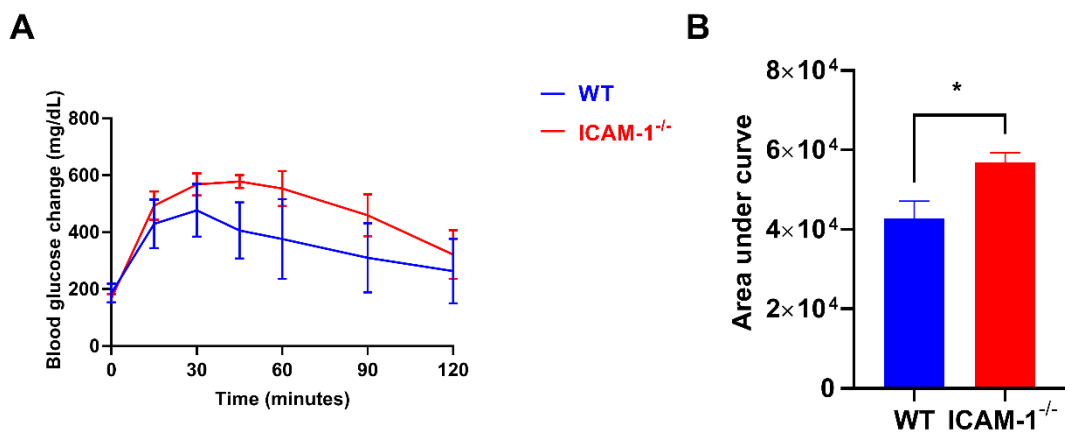
Figure:

Figure: ICAM-1 deficient mice exhibit more pronounced features of the metabolic syndrome. (A) Intraperitoneal glucose tolerance test and (B) corresponding quantification as area under curve of WD-fed WT (n = 8) and ICAM-1^{-/-} (n = 8) mice (p < 0.05).

P04-05 Carvedilol modifies metabolomics of bile acids in mice: effect of non-alcoholic steatohepatitis

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Background and aims: Carvedilol, a nonselective beta-adrenoreceptor antagonist, showed potential in preventing liver fibrosis in various experimental models except non-alcoholic steatohepatitis (NASH). Carvedilol may induce cholestasis, but its effect on bile acid metabolomics was not studied, despite BAs playing a significant role in the pathogenesis of NASH. Therefore, the present study aimed to study the influence of carvedilol on the progress of non-alcoholic steatohepatitis and changes in the homeostasis of bile acids (BAs).

Method: The NASH was induced in mice by a long-term high fat/high glucose/fructose diet, and BAs metabolomics was analyzed in the liver and intestine.

Results: Carvedilol administration to NASH mice reduced plasma activity of alanine aminotransferase and alkaline phosphatase and attenuated liver fibrosis. These effects were consistent with reduced collagen production in mice liver and in vitro in human hepatic stellate cells. Carvedilol also decreased the liver content of triglycerides by suppressing fatty acid synthesis via downregulation of stearoyl desaturase 1 in mice with NASH. The BAs content in plasma, bile, and feces was not significantly changed by carvedilol; however, the drug shifted BAs spectra toward more hydrophilic and less toxic alpha-muricholic acid and hyocholic acid. In contrast, carvedilol significantly increased plasma concentrations of BAs in healthy mice due to reduced trans-hepatocyte transport of BAs via downregulation of Ntcp and Bsep transporters.

Conclusion: We demonstrated that carvedilol attenuates the development of non-alcoholic steatohepatitis showing anti-steatotic and anti-fibrotic effects. Simultaneously, we uncovered a possible mechanism of the cholestatic effect of carvedilol, which is occasionally detected in clinical practice. Grant GACR 22-05167S supported the project.

P04-06-YI Dysregulation of the urea cycle enzymes determines a more severe NAFLD phenotype in diamond mice

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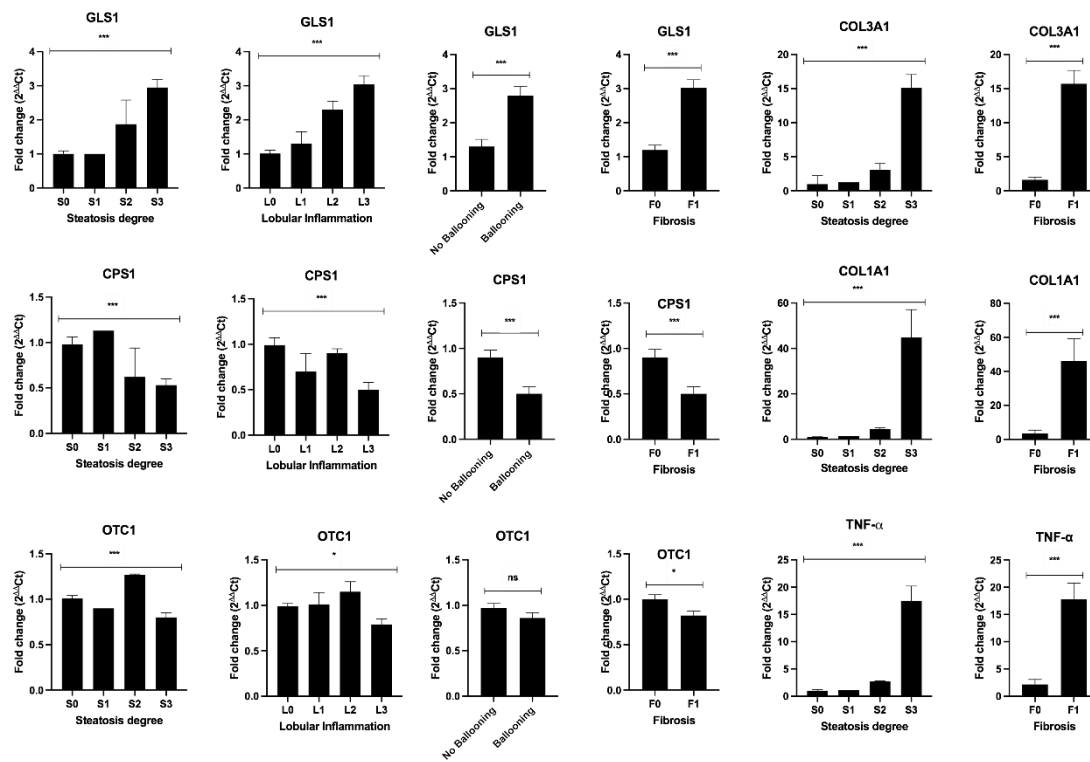
Background and aims: To evaluate changes in urea cycle enzymes (UCEs) in a DIAMOND preclinical animal model of NAFLD under different dietary interventions.

Method: DIAMOND mice (n = 28) were randomized to three dietary regimens: high-fat diet supplemented with fructose/glucose in drinking water (HF-HFD), choline-deficient high-fat diet model supplemented with 0.1% methionine (CDA-HFD) or standard diet, which was used as a control. HF-HFD and control animals were sacrificed after 21 or 30 weeks of diet intake. CDA-HFD animals were sacrificed after 12 weeks to avoid weight loss along with other adverse outcomes linked to more prolonged exposure to this type of diet intervention. Liver damage characterization includes well-established markers for fibrosis (collagen deposition and COL3A1, COL1A1, and α -SMA expression levels) and inflammation (TNF- α and IL-6 expression), together with body weight and standard biochemical measurements recorded each week or month, respectively. UCEs and glutaminase (GLS) expression levels were evaluated by qRT-PCR. Histological findings were evaluated by a blinded anatomopathologist.

Results: HF-HFD animals displayed signs of dyslipidemia (hypercholesterolemia), insulin resistance (higher glucose levels), and liver damage (raised AST and ALT levels) together with an increase in body and liver weight. CDA-HFD animals displayed similar AST levels, higher ALT levels and an increase in liver weight but did not show any of the other metabolic disturbances observed in HF-HFD animals. Both dietary interventions promoted a general upregulation of fibrotic markers (COL3A1, COL1A1, α -SMA, and collagen deposition) along with a significant upregulation of the pro-inflammatory marker TNF- α . Collagen deposition was found to be increased in both groups of animals, although did not reach statistical significance. Transcriptomic evaluation of UCEs revealed a significant downregulation in mRNA in carbamoyl phosphate synthetase-1 (CPS1) and ornithine transcarbamylase (OTC1) in both CDA-HFD and HF-HFD animals *versus* controls, together with an increase in GLS1 expression in all groups of animals, according to the different hepatic lesions, such as steatosis, ballooning, lobular inflammation and fibrosis stages (Figure 1).

Conclusion: DIAMOND mice, a preclinical model that mimics NAFLD, showed urea cycle dysregulation under different dietary interventions. These enzymes could play a key role in the development and progression of NAFLD.

Figure: UCEs and pro-fibrogenic and pro-inflammatory markers expression following different histopathological features.



P04-07-YI Impact of genes related to metabolic MAFLD in response to hypocaloric dietary intervention: nutrigenomic analysis

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Background and aims: MAFLD affects 25% of the world population and is the leading cause of liver transplantation. Hypocaloric diet has been shown to improve steatosis and NASH resolution and achieve regression of fibrosis. The impact of genetic polymorphisms on response to life-style intervention remains elusive.

Therefore, our aim will be to analyze the impact of polymorphisms in PNPLA3, MBOAT, TM6SF2 and HSD17B13 on hepatic and metabolic response after hypocaloric dietary intervention with Mediterranean Diet versus low-fat diet.

Method: Multicenter study of nutritional intervention in 61 obese patients with histologically proved MAFLD. The patients (29 women, 57 ± 13 years old, 42 with NASH, 19 with F3-F4, 31 with AHT, 17 with T2DM and 23 with dyslipidemia) followed a hypocaloric dietary intervention for 3 months (32 Mediterranean Diet and 29 low-fat diet). NASH resolution was assessed by *NASH resolution score* (Vilar-Gómez et al, Hepatol. 2016) and fibrosis improvement by transient elastography and *Fibrosis Improvement after Lifestyle Interventions* (FILI) (Vilar-Gómez et al, Liver Int. 2017). Patients were genotyped for previously described SNPs in the region of influence of the genes of interest by PCR with TaqMan probes. PNPLA3 (rs738409; C>G); TM6SF2 (rs58542926; C>T); HSD17B13 (rs6834314; A>G); MBOAT7 (rs641738; C>T). The impact of SNPs on delta improvement was analyzed.

Results: After the intervention, body mass index (35 ± 6.1 to 34 ± 6.4 kg/m²; p < 0.001), fibrosis (12 ± 7.1 to 9.4 ± 6.7 kpa; p = 0.001) and percentage of body fat (37.8 ± 7.6 to 34.3 ± 8.4; p < 0.001) improved significantly. NASH resolution was achieved on 19.7% of the overall cohort and fibrosis improvement on 31.1%. Weight loss was associated with protective genotype of TM6SF2 (CC). NASH resolution was not associated with any SNPs. Fibrosis regression was associated with genotype GG of PNPLA3. Finally, multivariate analysis-including sex, age, BMI and risk allele of each gene-demonstrated an independent association between risk allele for PNPLA3 and fibrosis regression (p = 0.006). No specific impact of genes was seen between these two types of diets.

Conclusion: Genetic variants related to MAFLD seem to impact on response to a life-style intervention with hypocaloric diet. PNPLA3 genotype GG was associated with better fibrosis regression. Genetic studies could improve the stratification of patients with MAFLD who are candidates for nutritional intervention.

P04-08 Functionalizing novel cancer related genes in liver disease and liver regeneration

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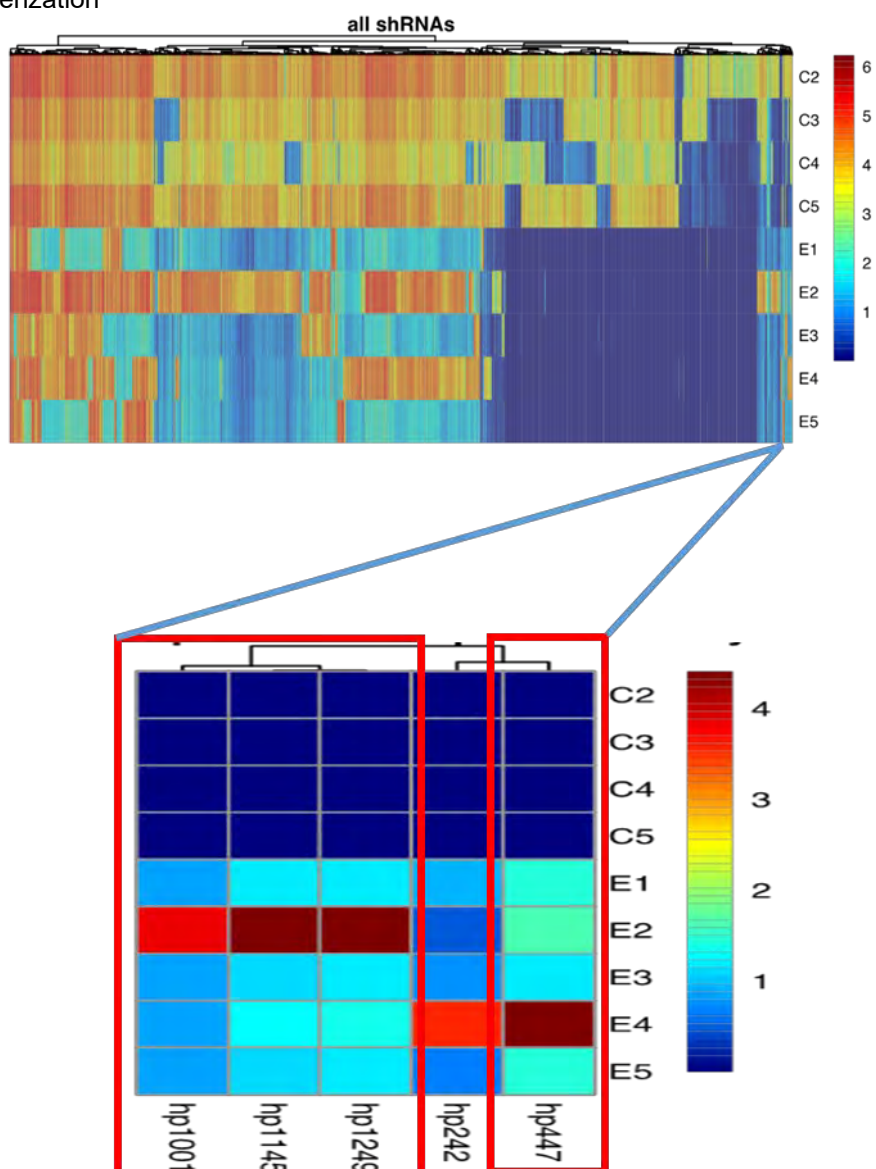
Background and aims: Caloric abuse and an inactive lifestyle have led to a widespread obesity and metabolic syndrome epidemic. Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are considered to be the hepatic consequence of it. As NAFLD/NASH condition are highly prevalent and drives a growing incidence rate of HCC, it is considered to be an important and alarming health issue. The critical question how the combination of an inflammatory microenvironment, created by NAFLD/NASH, abnormal metabolism and ongoing liver regeneration contributes to DNA instability and as a consequence promotes cancer is still unanswered. By this project we aim to reveal new relevant biomarkers for early and easier detection, treatment stratification and monitoring as well as approaches to therapies for both prevention and treatment in people who are at high risk for presenting fatty liver associated HCC to improve human health border.

Method: We take advantage of *in vivo* functional genomics screen in a mouse model of progressive fatty liver disease (Choline deficient high fat diet) to address this question. We screened a mir30 based shRNA library targeting 1000 genes, which human orthologs showed dysregulation in cancer. Scoring shRNAs will be validated *in vitro* as well as *in vivo* for influencing cell proliferation, survival and transformation and further will investigate the underlying mechanism with the goal for therapeutic intervention in disease progression.

Results: Computational analysis of shRNA abundance comparing CDHFD vs normal chow group shows selective enrichment for a number of targets. *In vitro* Knockdown of target genes show significant increasing in cell proliferation compared to control group. Short listed targets proceed for *in vivo* validation in NASH mouse model as well as *in vivo* liver repopulation and regeneration assay.

Conclusion: *In vivo* functional genetic screen identifies new regulators of NASH related liver cancer development.

Figure: RNAi screen under CDHF diet, significant enriched shRNAs selected for in detail characterization



P04-14 Decreases in liver cT1 accurately reflect therapy-induced histological improvements in NASH: a multi-centre pooled analysis

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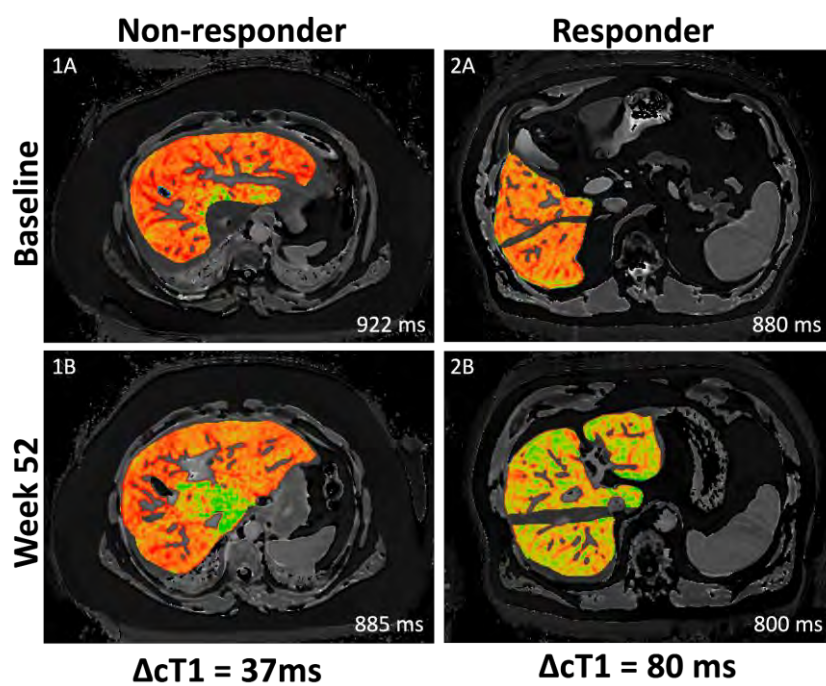
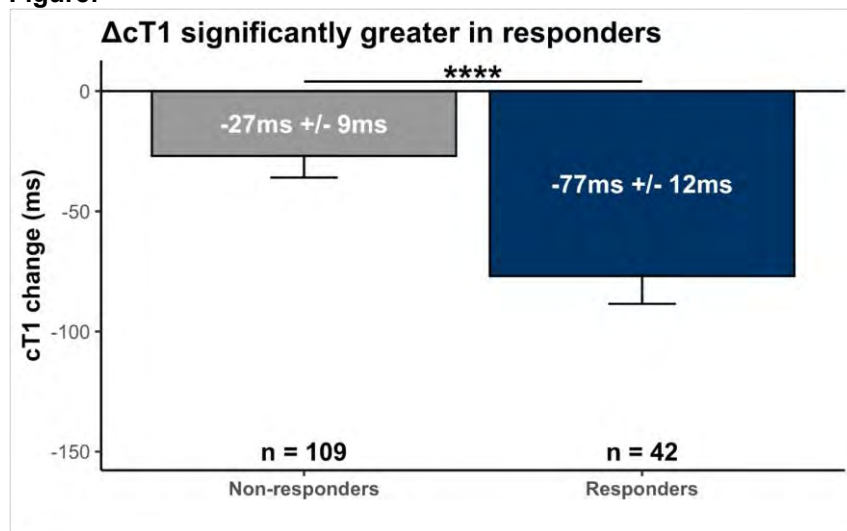
Background and aims: Current end points in non-alcoholic steatohepatitis (NASH) clinical trials require liver biopsy. MRI-derived biomarkers such as iron-corrected T1 (cT1) are less variable, risky, and costly alternatives. Previous analysis showed that a ~80 ms decrease in cT1 corresponds to 2-point decrease in the NAFLD activity score (NAS) and no worsening in fibrosis, indicative of clinically significant histological improvements, as stipulated by the FDA. Here, we validated this observation with data pooled from three interventional NASH studies.

Method: Study participants underwent MRI and biopsy at baseline and 12-52 weeks following intervention. Participants were characterized as responders (NAS decrease ≥ 2 with no worsening of fibrosis), or non-responders. Median Δ cT1 in the responders was calculated to determine a cut-off for detecting clinically meaningful changes in NASH. Diagnostic accuracy of Δ cT1 to identify responders was quantified using AUROC. Correlations between Δ cT1 and Δ histological markers were tested using spearman's rank.

Results: 151 patients from three NAFLD/NASH-confirmed cohorts were included. Change in cT1 correlated with change in NAS (R_s : 0.38; $p < 0.0001$) and was significantly higher in histological responders. Using Δ cT1 to identify responders resulted in an AUROC of 0.71 [0.62-0.81]. Δ cT1 of 77 ms corresponded to a two-point change in the NAS score.

Conclusion: The previously estimated change in cT1 of ≥ 80 ms was validated in a large independent data set with a clinically meaningful change in NASH corresponding to a Δ cT1 value of 77 ms in this pooled cohort. These results support the clinical use of cT1 for disease monitoring and its application as a surrogate end point for liver biopsy in clinical trials for NASH.

Figure:



P04-18 Proteomics and spatial transcriptomics show that ADAMTSL2 is a promising biomarker in NAFLD with significant fibrosis

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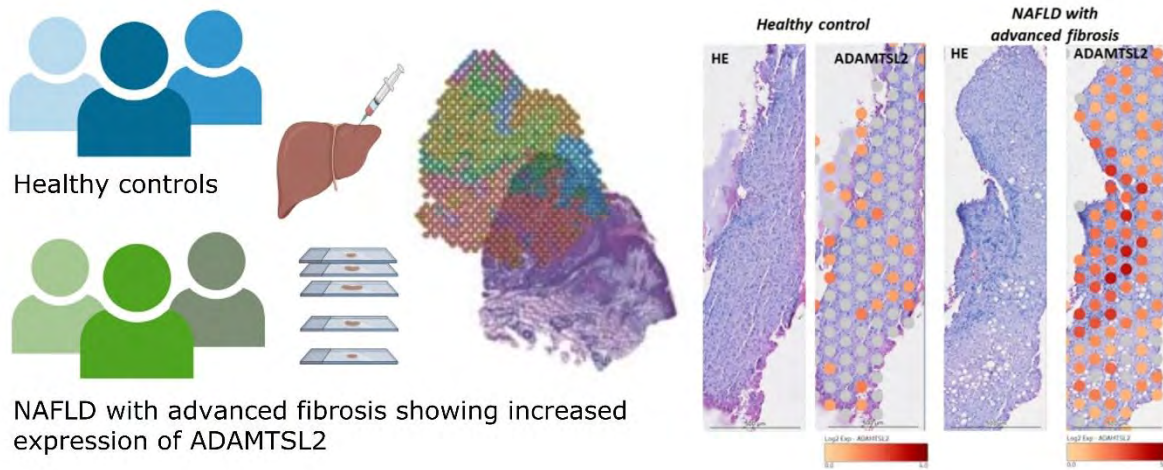
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Background and aims: The assessment of fibrosis is key to evaluate prognosis in non-alcoholic fatty liver disease (NAFLD). This study used the SomaScan Assay for multiplexed validation of candidate biomarkers and Spatial Transcriptomics (ST) in a prospectively collected cohort including participants with histologically conformed NAFLD and healthy controls. Our primary aim was to identify patients with significant fibrosis (F2-F4).

Method: The analyses included 193 NAFLD patients with fibrosis stage F0 n = 42, F1 n = 51, F2 n = 45, F3 n = 26, F4 n = 29 (31% with type 2 diabetes) and 79 healthy controls (26 with liver biopsies) from a prospective cohort study. Histology was assessed by two pathologists specializing in NASH. The serum proteome was analyzed using the SomaScan aptamer-based platform with 7k proteins (SomaLogic Inc., USA). ST were employed for in situ capturing allowing quantification and visualization of the transcriptome in liver biopsies (formalin-fixed paraffin embedded, FFPE). The Visium platform with 55 µm spot size (10x Genomics Inc., USA) was used and spatial mRNA expression was visualized by Loupe Browser 6 from the vendor.

Results: In patients with NAFLD, 20 proteins were increased (log (Odds) >1) in cases with significant fibrosis [-Log₁₀ P > 5]. The extracellular matrix glycoprotein ADAMTS Like 2 (ADAMTSL2) was clearly upregulated in significant fibrosis with an area under the curve (AUC) value of 0.818 (95% CI: 0.755-0.882), a sensitivity of 81% and specificity of 75% (optimal cut-off value of 2033 RFU). ADAMTSL2 gradually increased with fibrosis and differed between fibrosis stages [Kruskal Wallis test: P = 2.96-23] and between NAFLD patients and healthy controls. ST showed increased hepatic mRNA expression of ADAMTSL2 predominantly localized to areas with fibrosis and with increasing intensity in patients with significant fibrosis (Figure). Other proteins including ALDOB and FABP1 were increased in NAFLD but did not increase with fibrosis stage. Other proteins including FCMR and NOTCH3 were specifically upregulated in cirrhosis.

Conclusion: This study found that ADAMTSL2 appears to be associated with fibrosis progression and a promising biomarker to diagnose NAFLD patients with significant fibrosis. Other biomarkers were either increased in NAFLD (ALDOB and FABP1) and possibly associated with hepatic steatosis or were specifically upregulated in the cirrhosis stage (FCMR and NOTCH3). LC-MS/MS proteomics is currently being conducted on plasma and liver tissue in the same cohort to validate SomaScan data and to discover biomarkers that were not captured by the Somalogics platform.

Figure:

P04-20-YI Dysregulated wound healing contributes to the progression of post-transplant non-alcoholic steatohepatitis

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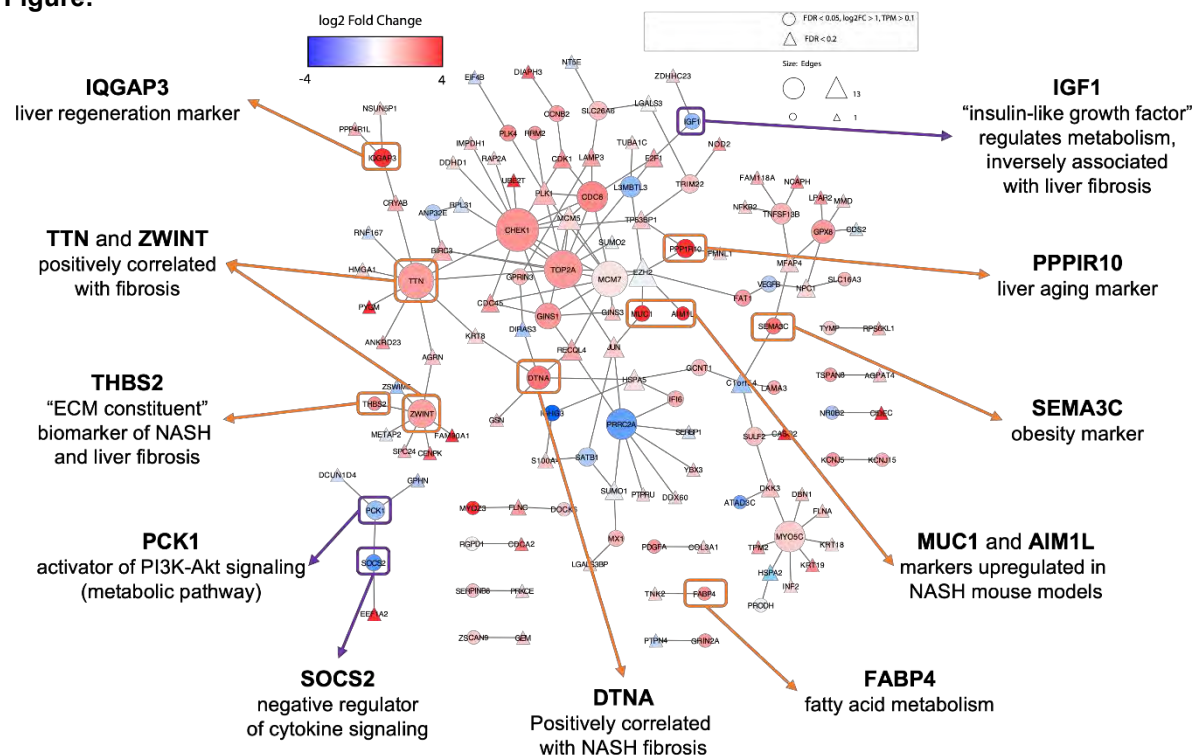
Background and aims: Non-alcoholic fatty liver disease (NAFLD) is on the rise alongside increase in obesity and diabetes. It is estimated that one in three North Americans have NAFLD. Advanced NASH, the inflammatory form of NAFLD, can cause liver damage leading to a necessity for liver transplantation (LT). NASH can recur or develop *de novo* at an accelerated rate presenting stage three fibrosis by five years after LT, reducing the benefit of the new graft. Thus, we aimed to examine the mechanistic basis for the aggressive post-LT NASH.

Method: The transcriptomes of post-LT liver biopsies presenting NASH, simple steatosis, or normal histology were profiled by RNA sequencing. Differential expression analysis was performed to identify significant gene expression changes between groups and compared to available non-LT NASH transcriptomes. Using ActivePathways, we identified enriched functional pathways and physical interactions of differentially expressed genes (DEGs) in post-LT NASH.

Results: We found 118 DEGs out of 19,847 measured genes in post-LT NASH. Our functional enrichment analysis showed significant transcriptomic changes in the PI3K-Akt pathway associated with metabolic alterations in NASH. Additionally, we identified nodes of protein-protein interaction consisting of published markers of NASH and liver fibrosis including MUC1, AIM1L, THBS2, IGF1, PCK1, and SOCS2. Significant changes in gene expression were also linked to regulation of wound healing, cell cycle, and fibrosis. Our comparison to non-LT NASH confirmed the increased activation of wound healing and angiogenesis pathways in the post-LT condition.

Conclusion: Our findings suggest the involvement of wound healing and fibrosis as a molecular basis for the accelerated development of fibrosis in post-LT NASH. Therefore, targeting mechanisms involved in liver fibrosis may be a therapeutic avenue for post-LT NASH to optimize graft survival.

Figure:



PPI network showing physical protein-protein interactions among DEGs in PT-NASH ($\log_2FC > 1$, $TPM > 0.1$; circles: significant DEGs ($FDR < 0.05$); triangles: less significant DEGs ($FDR < 0.2$))

P04-21 Expression dynamics of genes involved in lipid, glucose and xenobiotic metabolism after exposure to nuclear receptor ligands in 3D primary human hepatocyte spheroids

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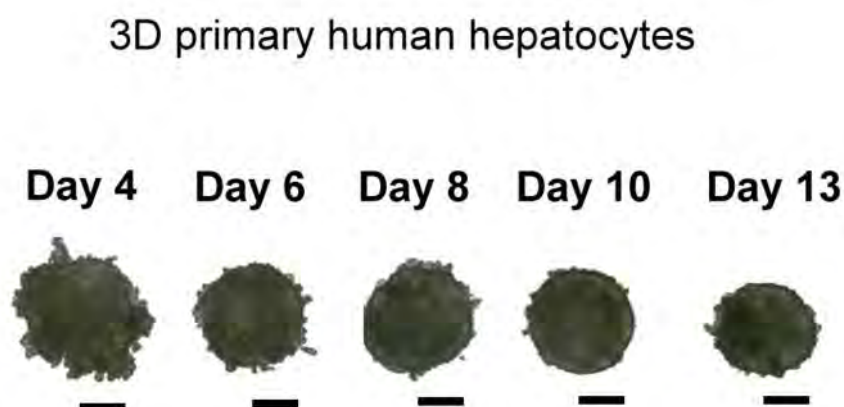
Background and aims: Nuclear receptors are critical in controlling transcriptome expression in hepatocytes through both endogenous signaling ligands and hormones or exogenous compounds. Although expression changes in lipogenic, gluconeogenic, xenobiotic-metabolizing, and bile acid synthetic genes have been described after activation with many NR ligands, the temporal dynamics of their expression is largely unknown due to limitation in primary human 2D hepatocyte cultivation.

Method: Recently, 3D spheroids of primary human hepatocytes (PHHs) have been characterized as the most phenotypically relevant hepatocyte model. We used 3D PHHs to assess time-dependent expression profiles of prototypic NR-controlled genes over the time course of 7 days. We mainly focused on ligands of Pregnane X (PXR), Farnesoid X (FXR), Constitutive androstane (CAR), and Vitamin D (VDR) receptors.

Results: We describe expression patterns for prototype genes controlling lipid and glucose homeostasis (FASN, GLUT2, G6PC, PCK1, PDK4), xenobiotic-handling genes (CYP3A4, CYP2C9, and MDR1), and bile acid and cholesterol metabolism (e.g. CYP7A1 and SHP) after treatment with agonists, antagonists or their combinations. We describe bell-shaped or biphasic regulation, synergism, and antagonism in the regulation of the genes in 3D PHHs. In addition, we calculated half-lives of CYP3A4 and CYP2C9 mRNA under induced or basal conditions in 3D PHHs.

Conclusion: The study shows the importance of long-term time-expression profiling of NR target genes involved in lipid and glucose metabolism or in xenobiotic clearance in phenotypically stable 3D PHHs and provides insight into NRs function in the liver beyond our knowledge from conventional 2D human hepatocyte models. Funded by EFSA-CDN CZ.02.1.01/0.0/0.0/16_019/0000841.

Figure:



P05-04-YI Exploring the impact of the genetic PNPLA3 I148M variant on primary human hepatic stellate cells by using healthy and diseased 3D extracellular matrix scaffolds

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Background and aims: The I148M variant of the Patatin-like phospholipase domain-containing 3 (PNPLA3) protein is a well validated risk locus for the human hepatic stellate cells (hHSCs)-driven fibrogenic progression of chronic liver diseases, particularly in NAFLD. In this study we investigated the impact of PNPLA3 I148M mutation on hHSCs by using transcriptomic data from hHSCs and an established cohort of patients with NAFLD, and validated findings in 3D culture models.

Method: RNAseq was performed on primary hHSC plastic-cultured and on biopsies from livers of 125 obese individuals, where both sets were genotyped for PNPLA3 I148M variants CG/GG. hHSCs CC/CG-PNPLA3 were cultured on 3D decellularized scaffolds from human healthy and cirrhotic liver with/without TGFB1 or CytosporoneB treatment. QRT-PCR, Seahorse, Western blot, NanoString, and cytochrome-c-oxidase activity (COX) assay was performed.

Results: Transcriptomic analysis on liver biopsies genotyped PNPLA3 CC/CG were compared with data from 2D cultured primary hHSCs PNPLA3 CC/CG. The comparison highlighted shared dysregulated pathways related to mitochondrial function, antioxidant response, ECM remodelling, the activated upstream regulator TGFB1 and its endogenous inhibitor, NR4A1. Dysregulation of analogous pathways was further confirmed by culturing hHSCs PNPLA3 CC/CG in 3D models of healthy and cirrhotic scaffolds. NanoString analysis showed a marked upregulation in the "Oxidative stress" pathway by cirrhotic ECM compared to healthy ECM. Mitochondrial dysfunction in CG-PNPLA3 cells was confirmed by OCR, quantified by Seahorse, and linked to a lower activity of COXIV. Moreover, mitochondrial antioxidant enzyme SOD2, NRF2 and CYGB expression was significantly lower in CG-PNPLA3 hHSCs, while ROS secretion was increased and higher in cells cultured in cirrhotic scaffolds indicating ECM-dependent effects. Similarly, TGFB1 and COL1A1 protein expression/secretion was significantly increased in CG-PNPLA3 hHSCs, and higher in cells grown on cirrhotic scaffolds. NR4A1, counteracting TGFB1 profibrotic effects, was lower and strongly inactivated by phosphorylation in CG-PNPLA3 cells, and further decreased by TGFB1 treatment in cirrhotic scaffolds. Treatment with CytosporoneB, a NR4A1 agonist, increased total NR4A1 in CG-PNPLA3 hHSCs cultured on healthy but not cirrhotic ECM.

Conclusion: Data from transcriptomic analysis on liver biopsies genotyped for PNPLA3 CC/CG and an *in vitro* hHSCs 3D-model recapitulating the healthy and cirrhotic liver microenvironment, showed a PNPLA3 I148M-driven disrupted mitochondrial function and antioxidant response, accompanied by increased TGFB1 signalling dampening the antifibrotic NR4A1 activity. Importantly, these features were exacerbated on cirrhotic ECM, highlighting the role of the fibrotic microenvironment in the progression of chronic liver diseases.

P05-07 Target metabolomics reveals the inhibition of the bile acid alternative pathway promotes the development of non-alcoholic steatohepatitis in mice

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases worldwide, which can progress to non-alcoholic steatohepatitis (NASH), even cirrhosis and hepatocellular carcinoma (HCC). Bile acids (BAs), as signal molecules, have shown a close relation with NASH, while the underlying mechanism remains unclear. There are two distinct pathways for BAs synthesis and metabolism, including the classical pathway producing the 12 α -hydroxylated primary BA (12-OH BA), eg. cholic acid (CA), and the alternative pathway producing the non-12 α -hydroxylated BA (non12-OH BA), eg. chenodeoxycholic acid (CDCA). Here, we characterized the 15 kinds of BAs profiles, especially the non12-OH BA pool of mouse with NASH. We aimed to uncover the relationship between NASH and non12-OH BA and be benefit to NASH diagnosis and therapy.

Method: We used western diet-fed C57BL/6 mice and compared to their controls fed with a normal diet for 10 weeks. Liver samples were fixed by 4% formaldehyde solution and embedded in paraffin, subjected to stained with hematoxylin and eosin (HandE) and Sirius Red for assessment of liver histology and fibrosis, respectively. The frozen liver samples were sectioned and then stained with Oil Red O for analyzing lipid accumulation. We determined plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bile acids (TBA) by the automatic biochemical analysis instrument. 15 bile acids in plasma and liver were quantified using liquid chromatography-mass spectrometry (LC-MS/MS).

Results: The biochemical analysis and histopathology assessment of livers showed that the mouse models of NASH were established successfully. Target BA metabolomic analysis revealed mice with NASH had higher concentrations of total BAs in their plasma while lower BA concentrations in liver tissue, compared with that of controls. Notably, the concentrations of 12 α -hydroxylated BAs, especially deoxycholic acid (DCA), and the ratio of 12 α -hydroxylated to non-12 α -hydroxylated BAs were significantly higher in the plasma with NASH mouse. Consistently, the concentrations of non-12 α -hydroxylated BAs, mainly alpha muricholic acid (MCA) were strikingly lower in the liver of mice with NASH. Hence, the alternative bile acid pathway may be inhibited by the western diet. Therefore, dysregulated BA metabolism, especially the down-regulated non-12 α -hydroxylated BAs, contributes to the development of NASH in mice.

Conclusion: In mouse models of NASH, BA composition especially non-12 α -hydroxylated BAs is profoundly altered, which indicates the inhibition of the alternative bile acid pathway. Our findings may open new insights for western diet-induced liver injury in-depth investigations, as well as provide a reference basis for more therapeutic drug mechanism research.

P05-08-YI Immune modulation by RIPK3 in NAFLD progression towards hepatocellular carcinoma

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Background and aims: RIPK3 is a well-established key executor of necroptosis, while its role in triggering the NLRP3 inflammasome remains poorly explored. NLRP3 inflammasome is an innate immune system sensor that plays a central role in all stages of carcinogenesis. In turn, the precise role of RIPK3-dependent signalling in hepatocarcinogenesis and immune response remains elusive. Here, we aimed to investigate the impact of blocking RIPK3 in liver carcinogenesis and tissue microenvironment.

Method: Two-week-old male C57BL/6 wild-type mice (WT) or *Ripk3*-deficient (*Ripk3*^{-/-}) pups were injected with diethylnitrosamine (DEN; 25 mg/kg i.p.), followed by feeding with a choline deficient-high fat diet (CDHFD) or a standard diet (SD) from 4 to 42-weeks-old. In parallel, mice were fed from 4 to 57-weeks-old with CDHFD. The liver was removed and macroscopic tumours were counted and measured for phenotypic characterization. Gene expression analyses were performed to evaluate markers of inflammation, fibrosis and infiltrated immune cells.

Results: Macroscopically discernible tumours were only detected in mice treated with DEN and DEN+CDHFD. Ablation of *Ripk3* diminished tumour frequency in both models, while also reducing the tumour size in the DEN model. In addition, *Ripk3* deficiency reduced hepatic infiltration of macrophages and expression of inflammatory markers in both DEN-treated and CDHFD-fed mice. Regarding adaptive immune mediators, the ratio of CD4⁺T to CD8⁺T cells was lower in DEN+CDHFD than in DEN livers in both WT and *Ripk3*^{-/-} mice, consistent with an impairment of the immune system. Nevertheless, *Pd-1* was globally reduced in *Ripk3*^{-/-} mice, compared with WT counterparts, except in tumours from DEN+CDHFD mice. This was accompanied by a general decrease in the expression of *Nlrp3* and its downstream effectors in pyroptosis, Caspase-1 and *IL-1 β* , in *Ripk3*^{-/-} mice. Strikingly, PD-1 levels were also reduced in mice lacking *Ripk3*, particularly in the DEN+CDHFD model.

Conclusion: *Ripk3* deficiency reduced the hepatic tumour burden associated with both chemical carcinogenesis and NAFLD-driven hepatocellular carcinoma. This was accompanied by changes in the infiltration of immune cells and their inflammatory profile. In particular, our results indicate that *Ripk3* deletion impacts on PD-L1/PD-1 axis, likely by impairing NLRP3 inflammasome activation, which could dampen T cell exhaustion in the liver microenvironment.

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P05-11 A global survey of health care professionals' awareness of non-alcoholic fatty liver disease

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Background and aims: Despite rapidly increasing prevalence, health care providers' knowledge about non-alcoholic fatty liver disease (NAFLD) may be limited. We assessed knowledge about NAFLD and associated factors among health care professionals of different specialties globally.

Method: Surveys concerning knowledge about NAFLD, consisting of 21 questions in 3 domains (epidemiology/pathogenesis, diagnostics, and treatment), were completed by health care professionals (physicians, physician assistants, advanced practice nurses, registered nurses, dieticians) from 88 countries from all continents. 622 surveys were completed over a period of four months.

Results: 80% of respondents were physicians (affiliation: 16% general practice (GPs), 34% internal medicine, 36% cardiology, 8% hepatology, 18% endocrinology, 7% gastroenterology, 2% oncology, 3% surgery, and 10% others) and originating from: 67% Europe, 1% North America, 8% Central and South America, 7% North Africa and Middle East, 2% Central and South Africa, and 16% Southeast Asia. Overall, the prevalence of NAFLD was estimated correctly by 36% of the responding physicians, and correct responses were significantly lower in physicians affiliated with general practice (33%) and cardiology (28%) compared to hepatology (74%).

In total, 91% of physicians reported to be familiar with the signs and symptoms of NAFLD, and 82% reported to be aware of the diagnostic criteria. Overall, 63% of physicians reported feeling comfortable diagnosing NAFLD and 68% reported feeling confident managing the disease.

Physicians affiliated with hepatology reported the highest confidence diagnosing and managing NAFLD (95% and 95% respectively). In contrast, physicians affiliated with cardiology reported lower confidence levels (54% and 52% respectively).

Overall, 57% of physicians who reported an incorrect prevalence rate, were confident in their diagnostic skills for NAFLD. Of those incorrect responders, 62% of those affiliated with general practice were still confident in their diagnostic skill compared to 73% and 66% affiliated with internal medicine and endocrinology, respectively. Correctly estimating the prevalence as well as confidence level of NAFLD management were independent from either practice location or continental region.

Among all respondents, physicians reported correct prevalence rate significantly more often than other health care professional (nurses, physician assistants and paramedics), although those physicians affiliated with general practice and cardiology separately, did not differ from other health professionals.

Conclusion: Physicians feel more comfortable diagnosing and managing NAFLD than their knowledge about the disease would suggest. Despite the growing burden of NAFLD, a significant knowledge gap remains for the identification, diagnosis, and management of NAFLD.

P05-14 Modelling clinical effect of patatin-like phospholipase domain containing 3 mutation in hepatocytes on severity of disease progression in 3D human NASH model

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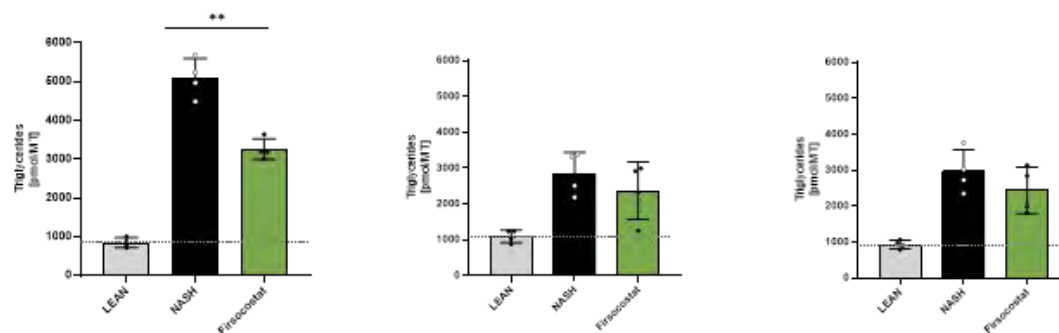
Background and aims: Non-alcoholic steatohepatitis (NASH) is a progressive severe disease characterized by lipid accumulation, inflammation and fibrosis in the liver. Single nucleotide polymorphisms (SNPs) at specific loci have revealed differential propensity to develop NASH. Among them, rs738409 located in patatin-like phospholipase domain containing 3 (PNPLA3), is highly frequent (30-50%) and results in a I148M amino acid change. PNPLA3 is a triacylglycerol lipase localized in lipid droplets but its function in the context of NASH is not fully understood and yet represents an interesting therapeutic target. The aim of this study was to investigate the effect of PNPLA3 I148M mutant on the development of NASH hallmarks in a 3D human liver *ex-vivo* culture model.

Method: Human 3D NASH model of spheroidal, scaffold free co-culture was developed of single donors of primary hepatocytes, Kupffer cells, liver endothelial cells and hepatic stellate cells. The hepatocytes were either from both wild-type or PNPLA3 I148M mutant. Upon exposure to defined lipotoxic and inflammatory stimuli such as free fatty acids and LPS in media containing high levels of sugar and insulin the 3D NASH model displayed key disease pathophysiological features within 10 days of treatment. Characteristic and quantifiable markers were established for anti-NASH drug efficacy testing such as triglyceride assays, secretion of pro-inflammatory cytokines/chemokines and secretion of pro-collagen type I/III.

Results: Thus, increase of intracellular triglyceride content as indicator of lipid accumulation and the secretion of inflammatory markers such as IL-6, MIP-1 α , TNF- α , IL-10, MCP-1 and IL-8 was observed in the NASH-treated as compared to control-treated wild type tissues. The increased fibril collagens deposition and secretion of procollagen type I and III peptides was detected under NASH conditions in wild type co-cultures. Importantly, treatment with anti-NASH drug candidates (Selonsertib and Firsocostat) affected biochemical end points indicative of disease progression and the results were to a large extent in line with clinical observations (Figure 1). 3D NASH tissues created with hepatocytes carrying the PNPLA3 mutation enhanced significantly the NASH model phenotype such as triglyceride levels and pro-collagen type I secretion as compared to the wild-type tissues.

Conclusion: In summary, using this 3D NASH model for drug candidates efficacy testing represents a promising approach for selection of the most effective drug candidates to move further in the development. Furthermore, we demonstrate that the 3D tissues with PNPLA3 I148M mutation in hepatocytes can enhance the overall NASH disease phenotype similar to the clinical situation.

Figure:
A



B

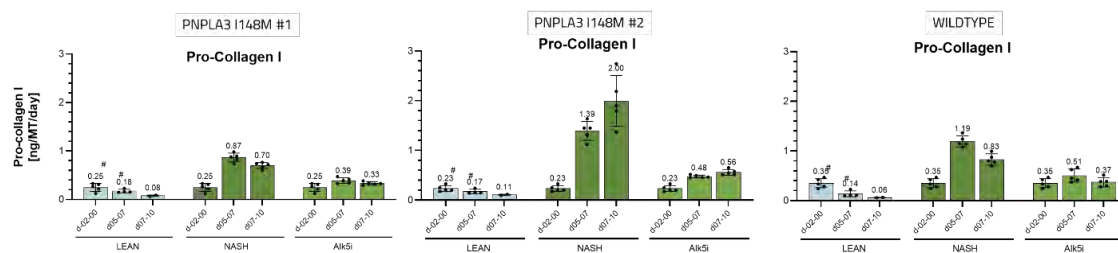


Figure 1. Biochemical and Fibrosis detection. A. Cellular triglycerides B. Pro-collagen I

P05-15 Single-nucleus ATAC-seq elucidates major modules of gene regulation in the development of non-alcoholic fatty liver disease

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Background and aims: NAFLD develops from NAFL to NASH during which multiple cell types may play different roles. Aiming to understand tissue composition of cell types, their gene expression and global gene regulation in the development of NAFLD, we performed single-nucleus and bulk ATAC-seq on the liver of rats fed with a high-fat diet (HFD).

Method: Male SHR/Izm strain rats were fed a normal diet or a high-fat atherogenic diet (24% fat, 15% protein, 5% cholesterol, 2% cholic acid). NAFL was caused by a HFD load from 12 weeks for 4 weeks, and NASH was caused by a longer term loading up to 8 weeks. Under the washout condition, where 4 weeks of HFD is followed by 4 weeks of a normal diet, NAFL was partially ameliorated. Along with rats only fed a normal diet (age of 16 or 20 weeks), five dietary conditions were examined. We performed single-nucleus ATAC-seq on one animal per dietary condition and bulk ATAC-seq on four animals per condition. By machine learning, we divided global gene expression into modules, such that transcription factors (TFs) in a module regulate a set of genes in the same module. For the discovered biological processes, we searched core genes, which were defined as genes central regarding co-expression and protein-protein interaction.

Results: In accordance with the pathological progression from NAFL to NASH that could have occurred between 4 and 8 weeks of the HFD intervention in our rat model, the proportion of inflammatory macrophages dramatically increased. In contrast, after 8 weeks of HFD, the proportion of hepatocytes largely decreased. In the washout condition, where 4 weeks of HFD was followed by 4 weeks of normal diet, the cell type composition returned similar to rats only fed with the normal diet. From the global analysis of TF binding and gene expression in single nuclei, we identified major modules of TF regulation, which could be shared between different cell types or be specific to certain cell types. One module shared among hepatocytes, endothelial cells, and macrophages was characterized by the binding of AP-1 TFs and a biological process of TNF α signaling via NF- κ B. For many of the discovered modules, TFs in a given module were known to regulate biological processes assignable to a set of genes in the same module, suggesting the validity of our module discovery algorithm. Finally, for the biological processes that emerged in TF regulation analysis, by incorporating known protein-protein interactions, we could identify core genes, many of which overlap with previously implicated NAFLD genes.

Conclusion: Using novel statistical methods, we elucidated a global picture of *in vivo* TF regulation in each cell type as a set of modules and discovered core genes for NAFLD-relevant biological processes.

P05-17 3D human NASH model as a screening-based discovery approach for selecting and prioritizing drug candidates

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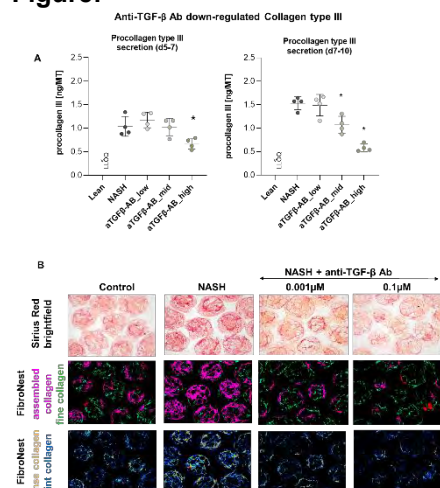
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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease, which includes hepatic steatosis that often progresses into steatohepatitis (NASH), characterized additionally by inflammation and fibrosis. We have modeled NASH using 3D human liver microtissues as a high-throughput tool for drug discovery. We present here a novel 3D human NASH model, which incorporates primary hepatocytes, Kupffer cells, liver endothelial cells, and hepatic stellate cells for high-throughput-compatible drug efficacy testing.

Method: We generated liver microtissues by culturing human primary hepatocytes, Kupffer cells, liver endothelial cells, and hepatic stellate cells in InSphero plates. Upon exposure to defined lipotoxic and inflammatory stimuli, including free fatty acids and LPS in media containing high levels of sugar and insulin, this 3D NASH model displayed pathophysiological hallmarks within 10 days of treatment. The accumulation of intracellular triglycerides (bioluminescent assay), secretion of pro-inflammatory cytokines/chemokines (Luminex), and pro-collagens type I and III (HTRF/ELISA) were measured. Quantification of fibrosis based on Sirius Red-stained tissue slices was performed using the FibroNest™ imaging platform.

Results: We observed increases in intracellular triglyceride content and the secretion of proinflammatory (e. g. IL-6, IL-1b, TNF-a) and profibrotic (e.g. IL-10, GRO-a, IP-10, MCP-1) cytokines/chemokines in the NASH-treated tissues as compared to the untreated controls. Further, we detected increased fibril collagen deposition, and increased secretion of procollagen type I/III peptides under NASH conditions. Whole transcriptome analysis of NASH-treated tissues versus control revealed activation of pathways and differential regulation of genes associated with lipid metabolism, inflammation, and fibrosis induction. Treatment with the anti-TGF-β antibody and ALK5i (TGF-βRI inhibitor) concentration dependently decreased secretion of pro-collagen type I/III (Figure 1). Decreased deposition of fibril collagens based on quantification of fibrosis of Sirius Red-stained tissues was observed in the presence of anti-TGF-β antibody and ALK5i. The efficacy results of drug clinical candidates Selonsertib and Firsocostat were in line with clinical observations.

Conclusion: In summary, this high-throughput and compatible 3D human NASH model represents a promising approach for NASH drug candidate efficacy selection early within the drug discovery process.

Figure:**Figure. Assessment of anti-fibrotic effects of anti-TGF- β Antibody.**

A) Anti-TGF- β antibody (Ab) treatment led to concentration-dependent decrease of procollagen type III, **B)** Sirius Red staining brightfield (BF) and phenotypic quantification of fibrosis (FibroScan) indicate increase of collagen fibrils deposition in NASH conditions vs control.

P05-18-YI Multiplex immunostaining identifies novel immune cell markers for non-alcoholic fatty liver disease and primary sclerosing cholangitis

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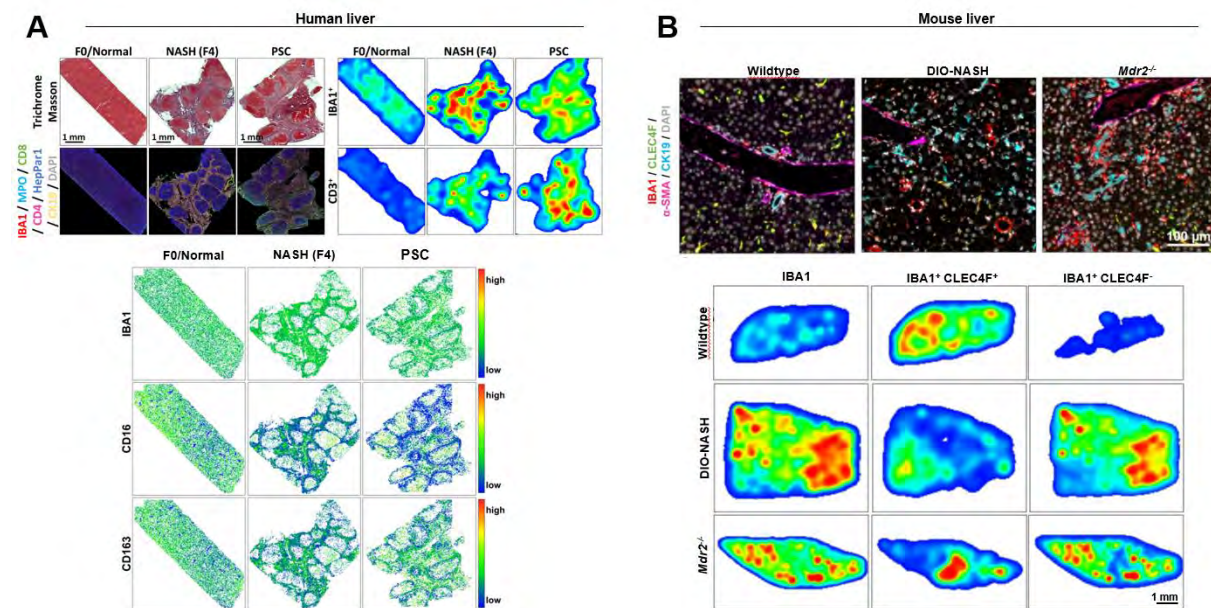
Background and aims: The progression of non-alcoholic fatty liver disease (NAFLD) towards non-alcoholic steatohepatitis (NASH) and of primary sclerosing cholangitis (PSC) is accompanied by hepatic infiltration of immune cells. This study aims to combine multiple novel analytic methods for the evaluation of disease stage associated histological changes, and for identifying similarities and differences between NAFLD, NASH, and PSC patients and corresponding mouse models.

Method: Human liver samples (total n = 85) from two independent patient cohorts and mouse samples from a diet induced obesity (DIO) model of NASH and Mdr2-deficient mice (total n = 35) were used. A specific, adapted sequential immunostaining method for hepatic parenchymal and immune cells was used on all human and mouse samples. Imaging cytometry and clinical or phenotypic data were combined for multidimensional analysis. Key findings on immune cell composition and phenotypes were validated in human clinical samples and functionally tested in mouse models.

Results: NASH and PSC disease stage was associated with significant loss of parenchymal areas, increased fibrosis and defined infiltration of myeloid and lymphoid cell populations. NASH patients predominantly exhibited myeloid cell infiltration, whereas PSC patients had a pronounced lymphoid cell response. Both diseases displayed intense ionized calcium-binding adapter molecule 1 (IBA1+) macrophage accumulation in the perlobular areas. Using a machine learning-based algorithm, IBA1 in combination with hepatocyte and ductular cell immunostaining predicted advanced disease stage in NASH and PSC. These findings were validated in mouse models of NASH and PSC. Our data revealed a drastic switch of the liver macrophage pool towards immature phenotypes with a distinct spatial pattern restricted to the perlobular areas.

Conclusion: Monocyte/macrophage infiltration and accumulation represents the dominant histological feature associated with the progression of NAFLD, NASH and PSC. Our findings show that IBA1, a pan-macrophage marker, is predictive of disease progression in human and can be used for the characterization of mouse models.

Figure:



P05-19 Tackling stages of fibrosis in metabolic-associated liver diseases with transcriptome profiles

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Background and aims: The prevalence of metabolic-associated fatty liver disease (MAFLD) is high, representing the most common form of chronic liver disease in the developed world with no indication of its decreasing. Patients with advanced stages of MAFLD have a high risk for co-morbidities of other pathologies, such as cardiovascular diseases, and obstructive sleep apnea. Biochemical mechanisms and pathways that would define a particular stage of MAFLD and predict the disease progression are still largely unknown, partially due to the multifactorial nature of the disease. Herein we applied transcriptome analysis and statistical modeling on the histologically determined MAFLD (fibrosis) stages of patients, to propose novel target genes that would better describe particular stages of MAFLD.

Method: 60 liver samples from an Italian cohort of morbidly obese patients who underwent bariatric surgery between 2014-2019 (discovery cohort) were stratified into different MAFLD stages of fibrosis (F0-F4) according to the Kleiner classification system. Patient data and clinical parameters were assessed. RNA was isolated from frozen liver samples and due to low RNA quality, we performed expression profiling by Affymetrix microarrays. Statistical analysis was performed with transcriptome analysis console software in order to decipher differentially expressed genes. Enriched pathways were assessed by KEGG and Reactome. A set of differentially expressed genes from our study was selected for validation in another group of MAFLD patients (78 patients, validation cohort), together with genes selected from studies Govaere et al. 2020 (PMID: 33268509) and Niu et al. 2022 (PMID: 35654907).

Results: In the discovery cohort we identified 9 differentially expressed, both known and novel candidate genes with FDR ≤ 0.05 , that can distinguish between MAFLD stages. *ITGBL1* and *ANKRD29* were identified as differentially expressed also in Govaere et al. study, showing a gradual increase with disease progression, while lumican, an extracellular matrix protein, was found to be significantly upregulated by Niu et al. These liver-expressed genes are currently researched in the validation cohort.

Conclusion: Despite a small discovery cohort and low quality of RNA from liver samples, we identified known and novel candidate differentially expressed genes that can distinguish between fibrosis stages of MAFLD. Of particular importance are liver genes whose transcripts or proteins can be identified also in the blood samples.

P05-21-YI Fatty hepatocytes induce skeletal muscle atrophy in vitro: a new three-dimensional platform to study the protective effect of albumin in non-alcoholic fatty liver disease

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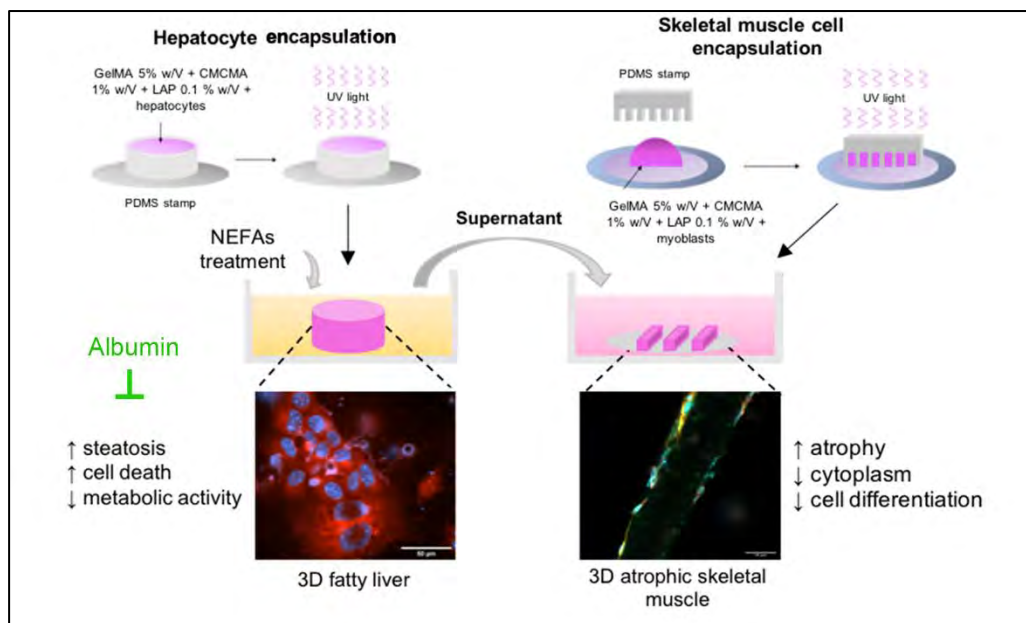
Background and aims: Non-alcoholic fatty liver disease (NAFLD), as the new silent disease of the 21st century, affects 1 in 4 people worldwide. It ranges from simple steatosis to non-alcoholic steatohepatitis, which may progress to cirrhosis and hepatocellular carcinoma. Many evidences in subjects with NAFLD show that the liver damage propagates to the skeletal muscle tissue leading to loss of skeletal muscle mass and/or physical performance, known as sarcopenia. Why fatty liver influences the development of sarcopenia in NAFLD is still not completely elucidated. Infusion of human serum albumin (HSA) has been demonstrated to reduce renal dysfunction, hospital readmissions and mortality in patients with acutely decompensated cirrhosis. HSA is synthesized in the liver and continuously secreted into the bloodstream, where it is the most abundant protein. It is the main transporter fatty acids and plays major roles in the binding of drugs and free radicals. In a previous work, we have demonstrated that hepatocytes cultured in vitro release albumin in response to lipid challenge for 48 hours (400 μ M non-esterified fatty acids (NEFAs)). Our hypothesis is that in NAFLD patients, the fatty liver induces gradual atrophy of skeletal muscle tissues accelerating the progression of liver disease that might be mitigated by administration of albumin.

Method: We developed a three-dimensional (3D) platform for hepatocytes and skeletal muscle cells crosstalk under NEFAs regimen. In this project, hepatocytes and myoblasts were encapsulated in a solution of gelatine methacryloyl, carboxymethylcellulose, and the photo-initiator LAP. The polymer was exposed under UV light for 30 seconds. The 3D tissues were fabricated using polydimethylsiloxane (PDMS) molds.

Results: 3D hepatocytes showed the typical signs of NAFLD such as lipid accumulation, metabolic activity impairment and apoptosis after 72h of culture with NEFAs mix. The 3D skeletal muscle cells incubated with supernatant from fatty hepatocytes displayed loss of cytoplasmatic mass, impaired metabolic activity, and altered gene expression involved in the maturation of myotubes and atrophy. In the following set of experiments, we pre-treated healthy hepatocytes with albumin prior to incubation with NEFAs, then we collected the supernatant and treated the skeletal muscle cells. We have seen reduced hepatocytes' lipids accumulation and signs of cell death, lower level of ammonia in the supernatant and improved muscle mass in the skeletal muscle cells.

Conclusion: This study establishes the direct connection between liver and skeletal muscle during the development of NAFLD, and the beneficial effect of albumin treatment on both liver and skeletal muscle tissue in an in vitro model of NAFLD. The tool herein presented can be employed as a customizable 3D in vitro platform for drug screening.

Figure:



P05-22 Sex-related differences of hepatic lipid metabolism and mitochondrial function in epileptic WAG/Rij rats: effect of early lipopolysaccharide challenge

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is associated with enhanced lipopolysaccharide (LPS) uptake by hepatic cells and its delayed clearance, leading to inflammation and oxidative stress (Carpino et al. 2020). Mitochondrial dysfunction plays a critical role in the occurrence of NAFLD (Mansouri et al. 2018), due to metabolic inflexibility in response to nutritional changes or inflammation (Annunziata et al. 2020). The association between seizure predisposition and peripheral and brain inflammation in a rat model of fatty liver disease was also evidenced (Aksoi et al. 2014). In epileptic WAG/Rij rats, hepatic oxidative and nitrosative homeostasis as well as lipid metabolism were markedly altered (Pirozzi et al. 2020). However, the relationship between NAFLD and LPS exposure in early postnatal days (PNDs) and the possible gender differences are still overlooked. Here, we evaluate the gender-related changes of hepatic lipid metabolism and associated mitochondrial dysfunction and oxidative damage in epileptic WAG/Rij rats, in presence or not of LPS challenge.

Method: At PND3, male and female WAG/Rij pups were injected with LPS (1 mg/kg, *i.p.*) and, at PND45, livers were collected, and mitochondrial respiration and beta-oxidation were polarographically detected. Hepatic lipid metabolism and oxidative stress (H₂O₂ release, reactive oxygen species production and lipid peroxidation as well as the mitochondrial activity of detoxifying enzymes) were evaluated by Western blot, Real-Time PCR, spectrofluorimetric and spectrophotometric analyses.

Results: In WAG rats, a more marked dysfunction of hepatic mitochondrial bioenergetics was shown in males than females, as demonstrated by decreased hepatic mitochondrial respiration, in presence of specific substrates, as well as the reduction of fatty acid oxidation. Interestingly, female rats were more susceptible to LPS not only for the reduction of hepatic respiratory capacity and altered coupling degree but also for endotoxin-induced hepatic oxidative damage, compared with no-treated females. Moreover, we showed a gender difference in the increase of hepatic lipid content and a compromised carnitine-palmitoyltransferase activity, a rate-limiting enzyme of fatty acid oxidation; these findings were associated with an altered expression of key genes and enzymes involved in the regulation of lipid metabolism including mitochondrial fatty acid transporters and key markers of steatosis.

Conclusion: Our study shed light on the relationship between epilepsy and hepatic damage, emphasizing sex-related mitochondrial alterations in epileptic rats, regardless or not of early inflammatory challenge, which can predispose to the development of NAFLD and associated disorders.

P05-23 *Anaerobutyricum soehngenii* as a next generation therapeutic microbe in a mouse model of non-alcoholic fatty liver disease

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Background and aims: The composition of microbes residing in the gut, known as the gut microbiota can affect the metabolic health of the host. By releasing metabolites or microbial components, gut microbiota have been proposed to affect the liver through the portal system. In this study, we investigated the effects of *Anaerobutyricum soehngenii*, a butyrate-producing and GLP-1 inducing anaerobic bacterium known to improve insulin resistance, as a next generation therapeutic microbe in a mouse model of non-alcoholic fatty liver disease (NAFLD).

Method: Mice were fed a western diet with 15% liquid fructose (WDF) to induce NAFLD and were compared to chow-fed control mice. The effect of WDF was evaluated by extensive analysis after terminating groups of mice after 8, 12, 16 and 20 weeks of feeding. In a subsequent experiment, mice fed WDF for 12 weeks were treated with *A. soehngenii* or placebo for 6 weeks. One week before termination, intraperitoneal insulin tolerance tests (ITTs) were performed. Liver histology scoring was done by a liver pathologist blinded to the grouping using the NASH-CRN scoring system. RNA was extracted from frozen liver tissues and sequenced on the Illumina Novaseq platform at a depth of 25 million reads per sample. Resulting transcriptomic data was analysed using DESeq and the KEGG pathway database.

Results: WDF feeding increased body weight and fasting blood glucose compared to control (both $p < 0.01$). In line, hepatic steatosis, inflammation and fibrosis scores increased throughout WDF feeding. Over time on the diet, the expression of hepatic genes in pathways related to cholesterol metabolism, PPAR signaling and inflammatory signaling increased. Genes that were upregulated over time on the diet, yet downregulated upon treatment with *A. soehngenii* included *lipin1* (*lpin1*), *fructose-bisphosphatase 1* (*fbp1*) and *interleukin 1 receptor type 1* (*il1r1*). Moreover, WDF-fed mice that received *A. soehngenii* had increased insulin sensitivity during ITT compared to placebo-treated WDF-fed mice ($p < 0.01$). However, liver histology scores were not affected upon *A. soehngenii* treatment.

Conclusion: WDF feeding robustly induces NAFLD in mice, both seen on histological evaluation as well as by the induction of genes in lipogenic, inflammatory and fibrotic pathways in the liver. Although no histological improvement of the liver was seen after treatment, *A. soehngenii* administration did result in improved insulin sensitivity and altered expression of several hepatic genes involved in gluconeogenesis, triglyceride synthesis and inflammatory signaling. We conclude that this high-fat diet with liquid fructose induces quite severe NAFLD and that *A. soehngenii* might be more beneficial in preventing than in treatment of disease.

P06-03-YI A co-micronized formulation of palmitoylethanolamide and phenolic compounds from olive leaves lessens hepatic dysmetabolic state in high-fat diet-induced obese mice

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Background and aims: The liver is crucial for the maintenance of normal glucose homeostasis. The development of non-alcoholic fatty liver disease (NAFLD) is considered an integral part of the insulin resistant state in obesity, diabetes and metabolic syndrome, contributing to the so-called gluco- and lipo-toxicity. Innovative pharmacological or nutritional approaches aimed at limiting the progression of NAFLD into non-alcoholic steatohepatitis and fibrosis are needed. The N-acylethanolamine palmitoylethanolamide (PEA) and some phenolic compounds from olive leaves, such as rutin and hydroxytyrosol (HT), have separately shown anti-inflammatory and metabolic effects. A new formulation containing PEA co-micronized with rutin and associated with HT is currently available in the EU market for dietary management of low-grade neuroinflammation in obese patients, namely Normast®3 (NORM3). This study evaluates the possible beneficial activity of NORM3 on the hepatic metabolic alterations in an animal model of high-fat diet (HFD)-induced obesity and related diabetes.

Method: Male C57Bl/6J mice were divided into 3 groups: a control group (STD) receiving standard chow diet; mice fed with HFD for 19 weeks; a HFD group administered NORM3 (PEA 10 mg/kg/die-Rutin 2 mg/kg/die, HT 0, 5 mg/kg/die *per os*) from week 12 up to week 19. Body weight was monitored throughout the experimental period. At the end of 7th week of NORM3 supplementation, the oral glucose and insulin tolerance tests (OGTT and ITT) were performed. Glucose and lipid metabolism in the liver were also evaluated by Western blot analysis and Real-Time PCR.

Results: Dietary integration with NORM3 reduced body weight of obese mice compared with non-treated HFD group and improved the glucose tolerance, as well as insulin sensitivity altered by fat overnutrition, as shown by OGTT and ITT, respectively. Then, we investigated the effect of NORM3 on the hepatic glucose and lipid dysmetabolism induced by HFD. NORM3 improved glucose homeostasis, as shown by i) increased phosphorylation of insulin receptor, ii) activation of protein kinase B (AKT) pathway, and iii) decreased gene expression of two main enzymes of gluconeogenesis, i.e., phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. Moreover, NORM3 reduced the transcription of key genes involved in the fatty acid oxidation, including carnitine palmitoyltransferase 1 and peroxisome proliferator activated receptor-gamma as well as its coactivator PGC1alpha. Finally, NORM3 counteracted the HFD-induced hepatic lipid accumulation, decreasing the mRNAs of transporter cluster of differentiation (CD36), known as a key marker of steatosis.

Conclusion: Taken together, our findings indicate a potential hepatoprotective effect of NORM3, proposing it as a new nutritional approach to counteract the impairment of glucose and lipid metabolism in NAFLD and diabetes related to obesity.

P06-08 Utilization of gluconeogenic glucose-6-phosphate via pentose phosphate pathway is increased in mice fed a high fat/high sugar diet compared to a high sugar diet alone

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Background and aims: In hepatocytes, the pentose phosphate pathway (PPP) is an important generator of NADPH for *de novo* lipogenesis (DNL) and for the maintenance of reduced glutathione in antioxidant defense. The development of non-alcoholic fatty liver disease (NAFLD) secondary to high sugar and/or high fat intake is characterized by elevated DNL rates and increased oxidative stress. It is not known whether PPP fluxes are modified in these settings. We developed a double tracer method that provides information on both DNL and PPP fluxes in feeding mice. We applied this to compare DNL and PPP fluxes in mice fed a normal chow diet supplemented with high-fructose corn syrup (HFCS-55) with a more obesogenic diet composed of high fat chow supplemented with HFCS-55.

Method: Twelve C57BL/6 mice were provided with standard chow (SC) with the drinking water supplemented with 30% (w/v) of HFCS-55 (55/45 mixture of fructose and glucose) for 18 weeks. Eleven mice were placed on high fat chow (HFC) with the same HFCS-55 supplement over the same period. At the beginning of the final evening, all mice were administered with 99% deuterated water containing and the HFCS-55 fructose component was enriched to 20% with [U-¹³C₆]fructose. On the following morning, mice were deeply anesthetized with ketamine/xylazine and sacrificed by cardiac puncture. Arterial blood was collected and centrifuged to isolate plasma, livers were freeze-clamped, and the samples were stored at -80 °C until further processing. Liver triglyceride and glycogen were purified and analyzed for ²H and ¹³C-enrichments by NMR. DNL rates were quantified from lipid ²H-enrichment and adjusted for liver triglyceride levels. Whole body adiposity was estimated from body water ²H enrichment. The fraction of gluconeogenic glucose-6-phosphate (G6P) utilized by the PPP was quantified from glycogen ¹³C-isotopomer analysis.

Results: Mice fed HFC + HFCS-55 has greater body weight (50 ± 1 vs 37 ± 1 g, $p < 0.0001$), higher whole-body adiposity (29 ± 1 vs $16 \pm 2\%$, $p < 0.0001$), and higher liver triglyceride levels (15 ± 2 vs 8 ± 2 g/100 g liver, $p < 0.025$) compared to those fed SC + HFCS-55. DNL rates were not significantly different between the two groups although the high-fat chow mice had a tendency for higher rates (1.02 ± 0.1 vs 0.75 ± 0.1 g/100 g liver, $p = 0.2109$). However, the fraction of gluconeogenic G6P utilized by the PPP was significantly higher for the high-fat chow mice (17.0 % *versus* 13.8%, $p = 0.034$).

Conclusion: A diet high in both fat and sugar is more obesogenic than a diet high in sugar alone. In the presence of high fat, DNL rates were sustained while PPP utilization of G6P derived from gluconeogenesis was significantly increased. This may be explained by additional demand for NADPH above and beyond that consumed by DNL, possibly in response to increased oxidative stress.

P06-09 A monoclonal antibody targeting non-junctional Claudin-1 inhibits fibrosis in patient-derived models by modulating cell plasticity

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Background and aims: Tissue fibrosis is a key driver of end-stage organ failure and cancer, overall accounting for up to 45% of deaths in developed countries. There is a large unmet medical need for anti-fibrotic therapies. Claudin-1 (CLDN1) is a member of the tight junction (TJ) protein family. While the role of CLDN1 incorporated in TJ is well established, the function of non-junctional CLDN1 (njCLDN1) is largely unknown. Aim of the study was to investigate safety and efficacy of targeting njCLDN1 for liver fibrosis treatment and cancer prevention in preclinical models.

Method: We developed a highly specific monoclonal antibodies targeting a conformation-dependent epitope of exposed njCLDN1. Using histology and transcriptomic, bulk and single-cell data, we characterized the CLDN1 expression in multiple cell types in healthy and diseased livers. We tested the biological and clinical relevance of targeting njCLDN1 in multiple mouse models of diet-induced liver fibrosis including a humanized diet-induced NASH model as well as patient-derived in vitro and ex vivo model. We studied the effect of targeting CLDN1 in models of lung and liver fibrosis and assessed the safety of a CLDN1 targeting monoclonal antibody in non-human primates.

Results: Our data show that CLDN1 is upregulated in liver tissue of patients with liver disease of all major etiologies and the level of CLDN1 expression is associated with fibrotic disease progression in patients with NASH.

We show, in patient-derived liver 3D fibrosis and human liver chimeric mouse models, that CLDN1 is a previously unknown mediator and target for liver fibrosis and cancer prevention. Targeting njCLDN1 reverted inflammation-induced hepatocyte pro-fibrogenic signaling and cell fate and suppressed the myofibroblast differentiation of hepatic stellate cells. Safety studies of a fully humanized antibody in non-human primates did not reveal any significant adverse events even at high steady-state concentrations. Antifibrotic effects in lung and kidney fibrosis models further indicate a role of CLDN1 as a therapeutic target for tissue fibrosis across organs.

Conclusion: Our results provide preclinical proof-of-concept for CLDN1-specific mAbs for treatment of advanced liver fibrosis and cancer prevention. These data pave the way for further therapeutic exploration of CLDN1-targeting therapies for fibrotic diseases in patients.

P06-10 A translational rat model to study metabolic associated fatty liver disease with fibrosis and portal hypertension

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Background and aims: The use of animal models is crucial to understand the underlying mechanisms in the onset and progression of metabolic associated fatty liver disease (MAFLD) and to develop novel therapeutic strategies. Moreover, the concept of MAFLD is not as restrictive as NAFLD and other potential causes of liver damage can coexist with metabolically-induced fatty liver and steatohepatitis (NAFLD), being alcohol consumption the most frequent liver injury in Southern European countries (MAFLD-OH). Although several animal models have been used in the field, there is an ongoing challenge to identify those models that best mimic human pathology to allow good translation of the obtained results into the clinics. We aimed to develop a dietary rat model that reproduces the full MAFLD phenotype observed in human disease, including features of the metabolic syndrome, steatohepatitis with liver fibrosis and portal hypertension.

Method: For model development, male Sprague-Dawley rats were fed with control diet (CD), high-fat high-cholesterol diet with glucose/fructose beverage (HFHC/GF) or the same diet plus 1-3% ethanol added to the GF drink (HFHC/GF-OH). The dietary model ended at 16 weeks based on the confirmation of fibrosis in liver biopsies performed on some individuals at week 12. By the end of week 16, liver hemodynamics, histology and metabolic parameters were characterized.

Results: Sirius Red staining revealed that the rats from HFHC/GF and HFHC/GF-OH groups developed perisinusoidal fibrosis by week 12. The HFHC/GF and HFHC/GF-OH livers also showed severe steatosis and inflammatory infiltrates. The diet intervention did not induce significant increments in body weight at any time point. However, at the end of the model (16 weeks), the liver to body weight ratio increased significantly more in the HFHC groups compared with the CD. Portal pressure (PP) significantly increased in both HFHC/GF and HFHC/GF-OH groups compared to CD group (13.29 and 11.98 mmHg vs 8.65 mmHg, respectively), secondary to a marked increase in intrahepatic vascular resistance (IHVR). No significant differences in PP and IHVR were found between HFHC groups. HFHC diet was associated with significant increases in fasting blood concentrations of AST, ALT, cholesterol, alkaline phosphatase, creatine kinase and albumin.

Conclusion: This translational animal model could become a suitable model for basic research of advanced stages of MAFLD and also for drug testing in this pathology.

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P06-11 Glutaminolysis and steatosis to steatohepatitis transition: role of glutaminase

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Background and aims: Glutaminolysis seems to play an essential role in the pathogenesis of NAFLD. The aim of this project is to study the role of GLS and its genetic regulation in an animal model of NAFLD and in a cohort of patients diagnosed by liver biopsy.

Method: Thirty-one male C57BL/6J mice were fed a HFHCC diet (40%fat, 1%cholesterol and 42g/L glucose/fructose) (n = 26) or chow diet (n = 5) for 52w. *Gls* gene expression, GLs activity and protein expression were assessed by qPCR, fluorometric (PicoProbe™ Activity Assay Kit, abcam) and IHC assays. Ammonia levels were determined in blood using ammonia kit (Arkray). Within a cohort of 110 biopsy-proven NAFLD patients we determined *GLS* expression, plasma glutamine and glutamate levels, and *PNPLA3*, *TM6SF2*, *MBOAT7*, *HSD17B13* genotypes. The length of a microsatellite in the promoter region of *GLS* was assessed by capillary electrophoresis (long allele = 14 GCA repeats or higher).

Results: An increase in *Gls* expression levels were found in NASH compared to control and steatosis liver of animal model (NASH Fold- 4.04 ± 1.33 ; $p = 0.019$ and $p = 0.033$, respectively). In addition, higher levels of *Gls* expression were found in the presence of ballooning ($p = 0.006$) and significant fibrosis ($p = 0.019$). A significant increase in GLS mitochondrial activity was found in NASH compared to control liver tissue (34.6 ± 5.7 vs 12.6 ± 1.9 mU/mg; $p = 0.015$), also correlating with protein expression by IHC ($p < 0.05$). Besides, blood ammonia levels were higher in NASH mice (81.2 ± 8.7 vs 36.2 ± 4.2 umol/L; $p = 0.001$).

In humans, we found higher *GLS* expression in the liver of NASH patients with advanced fibrosis compared to mild fibrosis (fold: 5.2 ± 4.4 ; $p < 0.05$; $n = 6$ /group). Plasma glutamate concentration (324 ± 94 vs 438 ± 216 uM; $p = 0.009$), and glutamate/glutamine ratio (0.9 ± 1.0 vs 4.1 ± 8.4 ; $p = 0.035$) were increased in NASH patients, reflecting greater glutaminase activity. Multivariate analysis demonstrated the length of the microsatellite and *PNPLA3* were associated with NAFLD phenotype (Table 1).

Conclusion: Glutaminase expression and activity are increased in NASH (animals and humans) liver samples, corroborating the role of GLS in the pathogenesis of NAFLD. The length of the microsatellite in the promoter of *GLS* was found to be a diagnostic factor of NASH, independently of previously known clinical/genetic factors. Further understanding of the regulation of GLS expression mediated by microsatellite length in NAFLD field is warranted.

Figure:

	Simple steatosis (29)	NASH (81)	Univariate	Multivariate
Age (years)	53.6 ± 13.3	56.3 ± 11.5	ns	
Sex (female, %)	41.4% (12)	56.8% (46)	ns	
BMI	31.5 ± 4.7	34.7 ± 7.4	0.009	0.011
T2DM (yes)	58.6% (17)	60.5% (49)	ns	
<i>PNPLA3</i> -GG	6.9% (2)	25.9% (21)	0.023	0.022
<i>MBOAT7</i> -T allele	51.7% (15)	75.3% (61)	0.018	
<i>TM6SF2</i> -T allele	20.7% (6)	19.8% (16)	ns	
<i>HSD17B13</i> -GG	0% (0)	9.9% (8)	0.079	
<i>msGLS</i> (long-long)	37.9% (11)	14.8% (12)	0.009	0.014

P06-14-YI Micro-IAlver: artificial intelligence-powered drug screening platform for 3D-bioprinted human liver microspheres

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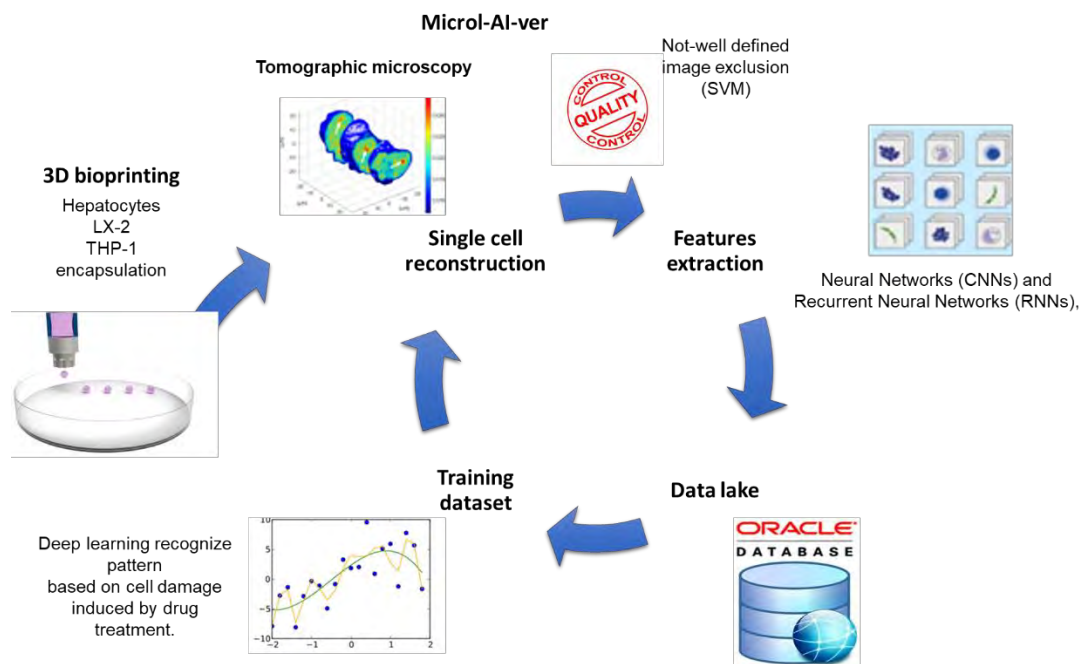
Background and aims: Non-alcoholic fatty liver (NAFL) is characterized by >5% of steatotic hepatocytes. Its more aggressive form is known as non-alcoholic steatohepatitis (NASH). This latter form is characterized by extended liver inflammation and often accompanied by fibrosis, which may progress to cirrhosis and hepatocellular carcinoma (HCC). The strongest predictor of mortality in NAFL/NASH patients is the advanced fibrosis. Currently, there are no drugs for NAFL/NASH treatment mainly because of the high failure rate of the clinical trial phases. The standard pipeline for the identification of drug candidates lacks accuracy and reproducibility, especially in the preclinical phase. Many in vitro models have been developed to recapitulate the pathophysiological conditions of NAFL/NASH in vitro. However, these models are difficult (i) to replicate, (ii) to scale to an industrial setting, and (iii) to identify a clear outcome. Here, we use *UniINK*, a patented three-dimensional (3D) printer ink employed for cell encapsulation. We used this technology to recreate a mini-liver using hepatocytes, hepatic stellate cells and Kupffer cells in a high-throughput manner, minimizing the input from the operator. Upon fat treatment, *UniINK* presents the main signatures of NAFL/NASH in a time-dependent manner, including the fibrosis. These features are identified, analysed, and interpreted by a machine learning approach to calculate the therapeutic index of drug candidates (microl-AI-ver).

Method: *UniINK* is collagen-based ink further crosslinked by tannic acid. HepaRG (human hepatocytes), LX-2 (human stellate cells), and THP-1 (human monocytes) are encapsulated in spheroids using 3D bioprinter and challenged with a mixture of oleic and palmitic acids. At the end of the experiment, the spheroids are passed through a flow-based tomographic microscope and interrogated one-by-one for features of hepatocyte's death, and HSC/THP-1 activation.

Results: We developed a robust 3D bioprinting method that fabricates 48k micro-livers/hour with an average cell density of 3M cell/spheroid at cost of €0.80/spheroids. The spheroids are incubated up to one month showing no necrotic area and high viability for untreated cells whereas fat and fibrosis accumulation and cell death in the fat treated condition. The features are classified using k-nearest neighbour (k-NN), support vector machine (SVM), and decision tree to build and implement the dataset. Deep learning networks, such as convolutional neural networks (CNNs) and recurrent neural networks (RNNs) are employed to automatically extract features, group the data, and recognize patterns to make decisions based on beneficial effects of drug treatment.

Conclusion: Microl-AI-ver allows: 1) to reduce the variability associated to the operator; 2) to be scalable at the industry level; 3) to spot early signs of fibrosis.

Figure:



P06-18 foz/foz mice with non-alcoholic steatohepatitis feature pathological cardiac hypertrophy

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is an umbrella term which describes different states of liver disease, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), cirrhosis and finally hepatocellular carcinoma. NAFLD case numbers are constantly rising for three decades with around one quarter of the global population being affected today. However, most fatalities among NAFLD patients are caused by cardiovascular disease (CVD), not by liver related events.

Left ventricular hypertrophy typically precedes the development of heart failure (HF). HF itself represents an important global health problem associated with high costs and high mortality.

Established pre-clinical animal models mainly focus on either NAFLD/NASH or CVD, yet; thereby neglecting possible reciprocal interactions between the liver and the cardiovascular system. Therefore, the aim of this study was to characterize cardiac alterations in *foz/foz* (FOZ) mice with NASH as foundation for future evaluation of potential links between liver disease and heart disease.

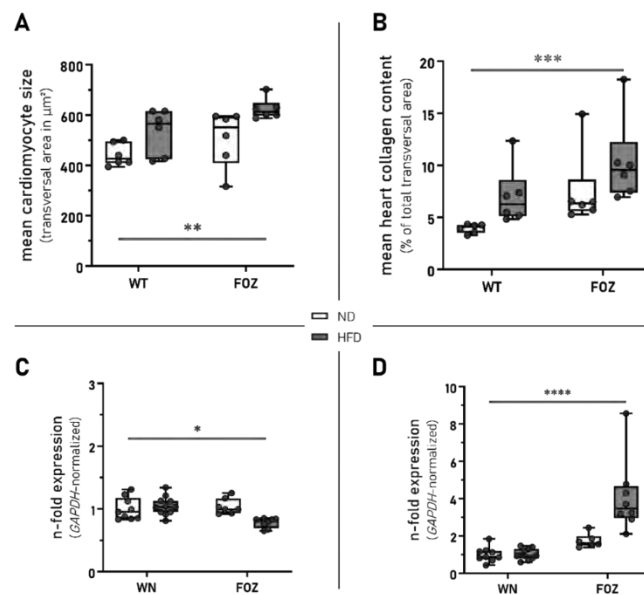
Method: Male FOZ mice and their age-matched wildtype (WT) controls were fed regular chow and high fat diet (60% kcal fat) for 24 weeks. Animals were then sacrificed, liver samples were gathered for histological assessment of the hepatic conditions, paraffin-embedded cardiac transversal cross sections were used to determine cardiomyocyte size (wheat germ agglutinin-staining) as well as cardiac fibrosis (Sirius Red-staining). Snap frozen tissue samples from left ventricular wall were gathered to evaluate the expression levels of marker genes for cardiac remodeling (*Myh6*, *Myh7*).

Results: FOZ mice on high fat diet (FH) featured all hepatic characteristics of progressive NASH (steatosis, ballooning, inflammation, fibrosis; NAFLD activity score = 8), whereas their WT littermates on normal diet did not show pathological hepatic alterations.

Compared to WT fed a normal diet, FOZ mice with NASH exhibited larger cardiomyocytes (A), increased collagen content (B) and a switch of myosin heavy chain gene expression in the heart, with downregulation of the predominantly postnatal isoform *Myh6* (C) and concomitant upregulation of the fetal variant *Myh7* (D).

Conclusion: *foz/foz* mice with NASH feature left ventricular hypertrophy with adverse cardiac remodeling characterized by enlarged cardiomyocytes, increased collagen built-up and a switch from adult to fetal gene expression pattern.

Figure:



P06-24 Anti-pd-1 treatment affects lipidomic profile in an animal model of NAFLD-HCC

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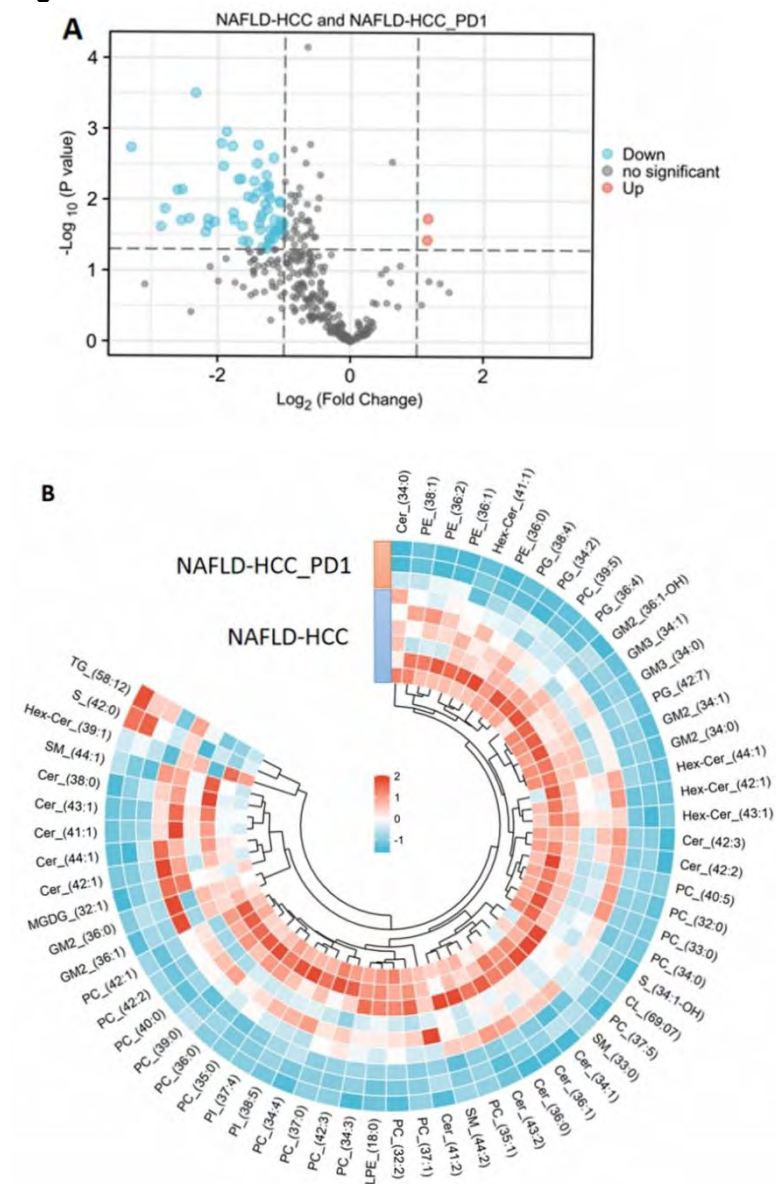
Background and aims: Non-alcoholic fatty liver disease (NAFLD) is considered a significant factor in the global increasing incidence rate of hepatocellular carcinoma (HCC). Drugs targeting the Programmed cell death 1 (PD1) hold promise as treatments for HCC. However, still little is known regarding the underlying mechanism of action. In this study, we aimed to explore whether anti-PD1 treatment affect the lipidomic profile in an animal model of NAFLD-HCC.

Method: 20 days-old wild-type mice were bred in a specific pathogen-free environment, received a single intraperitoneal injection of diethylnitrosamine (DEN, Sigma-25 mg kg⁻¹ body weight) at 20 days after birth to induce HCC and were subsequently fed with HFD at 4 to 6 weeks of age. After the 14 weeks of HFD-DEN induction, mice were randomly divided into two groups: 1) NAFLD-HCC_PD1 group, where NAFLD-HCC mice received the PD1 antibody treatment, 2) NAFLD-HCC group, where NAFLD-HCC did not receive any treatments. Lipids were extracted by the protein precipitation liquid extraction (PPLE) method and the lipidomic profile was then detected using liquid chromatography-mass spectrometry (LC-MS). Statistical analysis was run using the SPSS. Volcano plots were used to compare the differences between the two groups. The circle heat maps were used to visualize the final results.

Results: Overall, 3 mice were allocated to the NAFLD-HCC_PD1 group, while 6 mice in the NAFLD-HCC group. A total of 559 lipid metabolites were detected in each serum sample. The lipidomic profile from NAFLD-HCC_PD1 group was significantly different compared to the NAFLD-HCC (Figure 1A, $p < 0.05$). Specifically, in the NAFLD-HCC_PD1 group, 61 lipid metabolites were decreased, whereas 2 lipid metabolites were increased compared to the NAFLD-HCC (Fig 1A and 1B, $P < 0.05$).

Conclusion: In this study, we demonstrated that the PD-1 inhibitors affect the lipid profile in an animal model of NAFLD-associated HCC. The therapeutical effect of PD-1 inhibitors may be mediated by the modulation of the lipid metabolism.

Figure:



P07-06 miR-423-5p is associated with hepatic injury in a non-alcoholic fatty liver disease (NAFLD) preclinical model

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Background and aims: The expression of miR-423-5p in animal models under high fat diets (HFD) varies with liver damage. The main aim of this study was to explore the potential role of miR-423-5p in preclinical NAFLD model.

Method: Forty-four C57BL/6J male mice were included and randomized into two different diets: chow diet for 16w (n = 19) and choline-deficient, 0.1% methionine supplemented high-fat diet for 16w (n = 25). Within both groups, one arm of the study included animals treated with morpholine-based antisense probes against a miRNA of interest related to NAFLD (miRNA-X-3p). On the other hand, a control group was explored in order to evaluate the specificity of this antisense treatment (miRNA-X-5p). The hepatic expression of the microRNA miR-423-5p was evaluated by qPCR. Histopathological findings were evaluated by a pathologist blinded to the provenance of the samples and both NAS Score and liver fibrosis by Kleiner were calculated. SPSS v24.0 was used for the statistical analyses.

Results: In this study, we observed increased ALT levels in all CDA-HFD mice vs. controls, especially high in miRNA-X-3p group ($195.86 \pm 25, 38$ vs. 45.76 ± 14.71 UI/ml; $p = 0.002$). Besides, hepatic fibrosis was observed in CDA-HFD mice vs. controls (Figure 1A); as well as increased NAS Score rates (Figure 1B). NAS Score and liver fibrosis were found to be correlated ($r = 0.868$; $n = 44$; $p = 0.0001$). After sacrifice, a decrease in miR-423-5p was observed in those mice injected with the morpholine (miRNA-X-3p) versus control group under chow diet (fold change 0.31 ± 0.09 vs. 1 ± 0.15 ; $p = 0.005$) (Figure 1C). Similarly, in the CDA-HFD group a decrease in the expression of this miRNA was also observed in the morpholine group when compares to controls (fold change 0.64 ± 0.08 vs. 1 ± 0.09 ; $p = 0.021$) (Figure 1D).

Conclusion: miR-423-5p was downregulated in the animal model (both in chow and CDA-HFD groups) when injected with the morpholine and compared to controls. The expression of this miRNA was found to be decreased in parallel with the increase in liver injury evaluated by NAS Score. Therefore, epigenetic modifications can be considered a potential therapeutic target in NAFLD.

Figure:

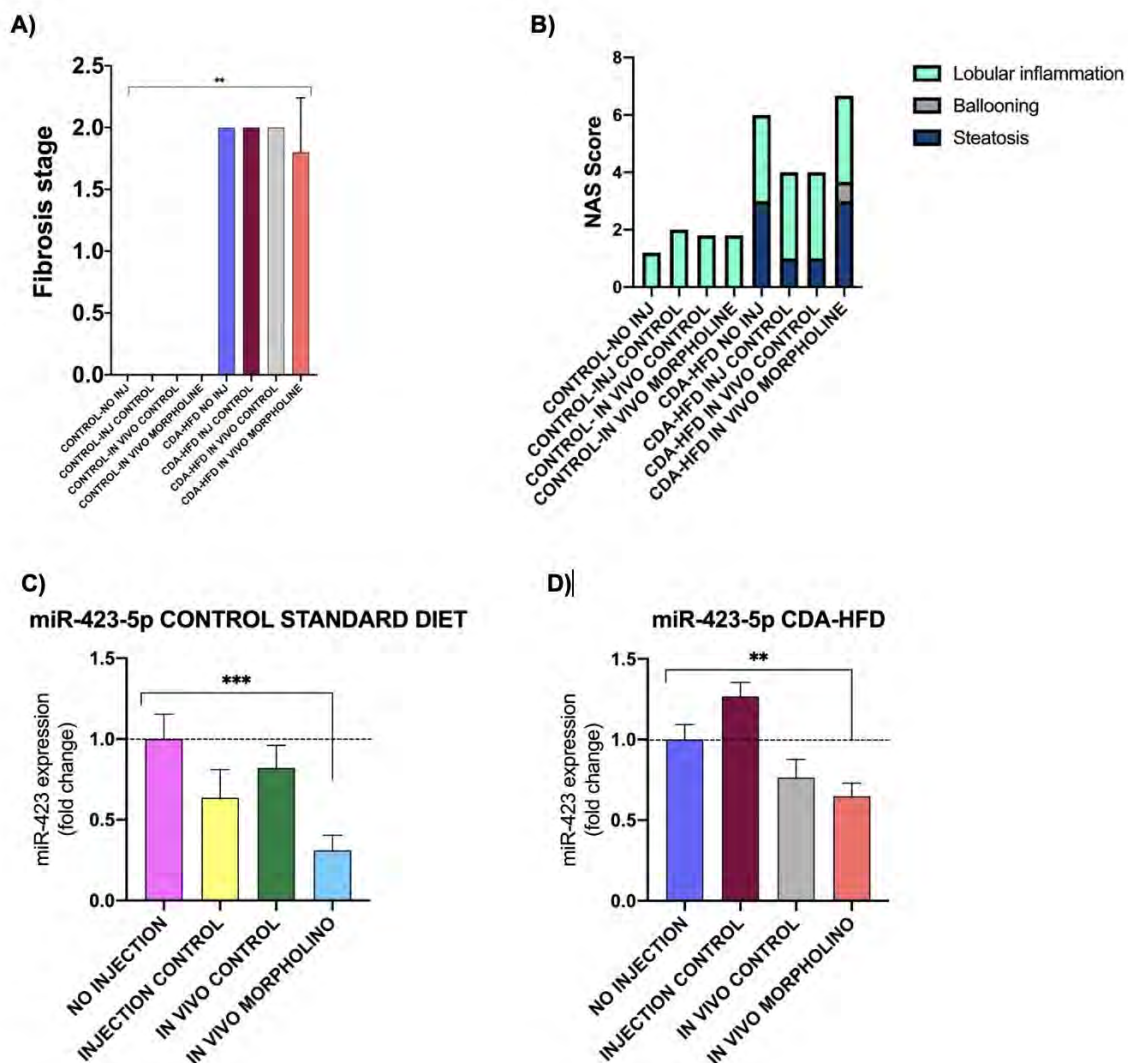


Figure 1. Hepatic Fibrosis and NAS Score in all mice (1A, 1B) and fold change of hepatic miR-423-5p in an animal model with two different diets: control (1C) and choline-deficient, 0.1% methionine supplemented high-fat diet during 16 weeks (1D).

P07-07 A specific lipidomic fingerprint is associated with the development of nafld-associated hcc in an animal model

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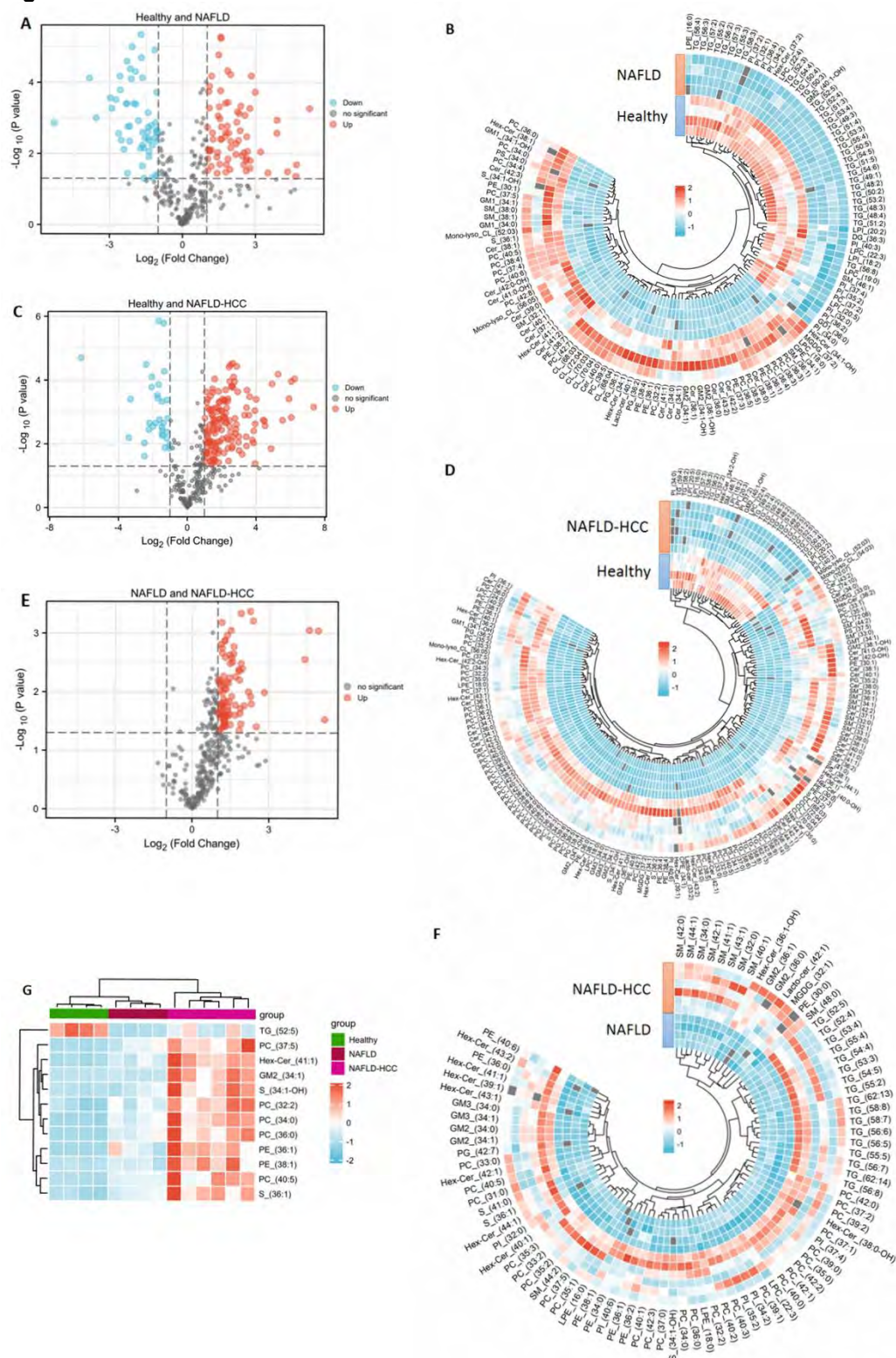
Background and aims: Non-Alcoholic Fatty Liver Disease (NAFLD) is a leading cause of liver disease worldwide and is still increasing. Hepatocellular carcinoma (HCC) represents an important complication of NAFLD. Yet little is known regarding the tumorigenesis in these patients. In this study, we aim to analyse the lipidomic profile in an animal model of NAFLD-associated HCC.

Method: 20 days-old wild-type mice were bred in a pathogen-free environment and randomly divided into three groups: 1) NAFLD group, where wild-type mice were fed with (high-fat diet) HFD at 4 to 6 weeks, 2) NAFLD-HCC group, where wild-type mice received a single intraperitoneal injection of diethylnitrosamine (DEN, Sigma-25 mg kg⁻¹ body weight) at 20 days after birth to induce HCC and were subsequently fed with HFD at 4 to 6 weeks of age, 3) control group. At 9 months, serum samples were collected. Lipids were extracted by the protein precipitation liquid extraction (PPLE) method and the lipidomic profile was then detected using liquid chromatography-mass spectrometry (LC-MS). SPSS was used for statistics.

Results: A total of 559 lipid metabolites were detected in this study. When the NAFLD (n = 4) was compared to the control group (n = 4), 127 lipid metabolites were significantly different between the groups (Fig 1A, p < 0.05), of which 72 lipids were increased and 55 were decreased in the NAFLD group. A valid model could separate NAFLD-HCC (n = 6) vs control group (Fig 1C, P < 0.05), showing that 145 lipids were elevated and 32 were reduced in the NAFLD-HCC group. 89 lipids were increased in NAFLD-HCC group compared to the NAFLD group (Fig 1E, P < 0.05). Finally, when the results from the three circle heat maps were compared, 12 lipid metabolites showed a consistent change when progressing from control to NAFLD and NAFLD-HCC group (Fig 1G).

Conclusion: In this study, we identified a specific lipidomic fingerprint which was associated with the development of HCC in an animal model with NAFLD. Lipidomic profile may provide further insight in the pathogenesis of HCC in NAFLD, as well as become a promising biomarker for detecting the disease.

Figure:



P07-09 Role of TGR5 in fat-to-liver communication through extracellular vesicles in the context of NAFLD

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is characterized by intracellular lipid accumulation in the liver and associates with pathogenic changes in other metabolic organs, particularly the adipose tissue (AT). In parallel, increasing evidence support a functional role for extracellular vesicles (EVs) in inter-organ crosstalk during NAFLD progression. Finally, activation of Takeda G-protein coupled bile acid receptor 5 (TGR5) was recently shown to ameliorate NAFLD in experimental mouse models.

Method: Here, we aimed to elucidate whether modulation or activation of TGR5 in adipocytes changes the release (and content) of EVs; and the functional role of such EVs upon stressed hepatocytes, *in vitro*. In parallel, we evaluated the role of AT TGR5 in a mouse model of NAFLD. AT-derived EVs were isolated from 3T3-L1 adipocytes exposed to lipopolysaccharide (LPS) and then incubated with unstimulated HepG2 hepatocytes. Alternatively, EVs were isolated from adipocytes after silencing of TGR5 or exposure to different TGR5 agonists-including bile acids-and then incubated with HepG2 cells stimulated with LPS. AT-derived EVs were isolated by polymer-based precipitation and characterized by nanoparticle tracking analysis. AT-specific TGR5 KO mice and respective littermate controls were fed a high-fat diet (HFD) for 14 weeks. Gene expression levels were analysed by qRT-PCR and immunoblotting.

Results: Results showed that AT TGR5 plays a key role in the metabolic response of mice to high calorie intake. In fact, AT-specific TGR5 KO mice fed the HFD displayed significantly increased body weight, compared to wild-type controls, starting at 9 weeks of feeding. EVs released from LPS-stimulated adipocytes triggered an inflammatory response in HepG2 cells, as seen by the increased mRNA expression of different inflammatory cytokines, resulting in enhanced cell death. Interestingly, incubation of unstimulated HepG2 cells with EVs isolated from TGR5-silenced adipocytes similarly increased the expression of inflammatory cytokines, while also promoting lipogenesis. Incubation of adipocytes with different TGR5 agonists activated critical intracellular signalling mediators. Further, EVs released from TGR5-activated adipocytes ameliorated the inflammatory response of HepG2 cells stimulated with LPS.

Conclusion: Overall, our results suggest that EVs from TGR5-activated adipocytes contain anti-inflammatory molecules capable of exerting a functional role within hepatocytes, with *in vivo* data underscoring the role of TGR5 in ameliorating obesity and intra-organ lipid accumulation. A better characterization of TGR5-mediated fat-to-liver inter-organ communication will elucidate the prospective therapeutic potential of TGR5 agonists for metabolic diseases (HR17-00601, Fat2LiverTGR5; and PTDC/MED-PAT/31882/2017).

P07-11 Telmisartan improves hepatic mitochondrial energy metabolism in mouse nutritional model of non-alcoholic steatohepatitis

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Background and aims: Telmisartan, a non-peptide antagonist of the angiotensin II type 1 receptor, was shown to reverse liver damage and fibrosis in non-alcoholic steatohepatitis (NASH). There is growing evidence that mitochondrial dysfunction plays an important role in the pathogenesis of NASH. In our previous experiments, we have found decreased total capacity of respiration and succinate-activated mitochondrial respiration in a mouse nutritional model of NASH. Therefore, the aim of the present experiment was to study the effect of telmisartan on mitochondrial energy metabolism.

Method: The NASH was induced in male mice fed a high fat/cholesterol diet and glucose/fructose syrup for 36 weeks. Last 6 weeks of the experiments, mice were administered daily telmisartan (oral gavage, 5 mg/kg b.w./day). Plasma liver enzymes, protein and mRNA expressions, liver histological changes and mitochondrial respiration of hepatic tissue homogenates (high-resolution respirometry-OROBOROS Oxygraph 2k) were assessed.

Results: Telmisartan reduced absolute and relative liver weight and visceral adipose tissue weight, activities of ALT and AST and steatosis grade. These effects were accompanied by improvements in total capacity of respiration (capacities of oxidative phosphorylation and electron transport system) and succinate-dependent respiration. An increase in succinate-dependent respiration was consistent with amelioration of expression of succinate dehydrogenase (SDH) subunits (A, B and D), SDH complex assembly factor 2 and succinate receptor 1.

Conclusion: We demonstrated that telmisartan reverses the development of NASH in mice. Along with the reversion of NASH, telmisartan significantly improves hepatic mitochondrial energy metabolism. The question remains whether telmisartan has a direct effect on liver mitochondria or whether it is mediated by an improvement of metabolic flexibility.

This work was supported by InoMed project CZ.02.1.01/0.0/0.0/18_069/0010046 co-funded by the European Union.

P07-13 Circulating Interleukin-32 levels are associated with arterial hypertension in individuals at risk of NAFLD

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is associated with metabolic comorbidities such as arterial hypertension and with an increased risk of cardiovascular disease (CVD), but the mechanism remains unclear. We recently reported that Interleukin-32 (IL32) is strongly upregulated in the liver of patients with severe NAFLD and secreted in the circulation, correlating with liver damage. Accumulating evidence suggests that IL32 affects endothelial function and may contribute to CVD. Aim of this study was to examine the association of plasma IL32 levels with metabolic comorbidities in healthy individuals with metabolic risk factors for NAFLD.

Method: IL32 plasma levels were determined by Human IL-32 DuoSet ELISA (R&D Systems) in the prospective Liver-Bible cohort 2020 (n = 948, age 53.9 ± 6.4 years, 83% males). Inclusion criteria were age 40-65 and ≥3 among: BMI ≥ 25 Kg/m², glucose ≥ 100mg/dl, triglycerides ≥ 150mg/dl, HDL < 45/55 mg/dl in M/F, impaired blood pressure control (systolic/diastolic > 130/80mmHg). All underwent metabolic characterization and non-invasive evaluation of liver damage by elastometry. Multivariable generalized linear models were fitted to analyze the independent determinants of log normalized IL32 levels.

Results: Among participants, 475 (49.3%) had NAFLD (CAP ≥ 275), 17 (1.8%) liver stiffness measurement (LSM) ≥ 8. Of these, 287 (30.3%) had very low circulating IL32 (<10pg/ml); median IL32 concentration was 559 pg/ml (IQR: 6258-4936pg/ml). High circulating levels of IL32 were associated with female sex (p = .0380), as well as impaired blood pressure control (p = .0024), detected in 71% of participants. Notably, IL32 levels were correlated with systolic blood pressure (p = .0174), but not diastolic blood pressure, and the use of some antihypertensive agents including beta-blockers and ace-inhibitors was inversely associated with IL32 (p = .0276 and p = .0499 respectively). Among other liver disease predictors, plasma IL32 resulted inversely correlated with HbA1c (p = .0103), but not with fasting insulin nor with circulating lipids nor with ferritin/CRP. The independent determinants of circulating IL32 levels are shown in the Table1.

Conclusion: Results suggest a potential cross-talk between IL32 and CVD in individuals with metabolic risk factors. Additional studies are warranted to examine the possible role of IL32 possible as therapeutic target to reduce NAFLD comorbidities.

Figure:

	Estimate	SE	p-value
Age	0.0029	0.0067	0.1886
Sex, F	0.1163	0.0561	0.0380
BMI	-0.0041	0.0136	0.7633
Hypertension, Yes	0.1268	0.0471	0.0072
Beta-blockers intake	-0.3030	0.1376	0.0276
ACE inhibitors	-0.1242	0.0634	0.0499
HbA1c	-0.0246	0.0103	0.0171

TABLE 1. Multivariable analysis for independent determinants associated with circulating IL-32 levels.

P07-16 Calorie restriction combined with metformin promote the remission of NAFLD

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is in the spotlight owing to its fast increase in prevalence worldwide. The hepatic lipid accumulation is a NAFLD hallmark, where triglycerides and cholesterol esters are stored in the lipid droplets. Calorie restriction (CR) is the only first-line treatment for early NAFLD (simple steatosis) but exists a lack of evidence about its effectiveness in NASH.

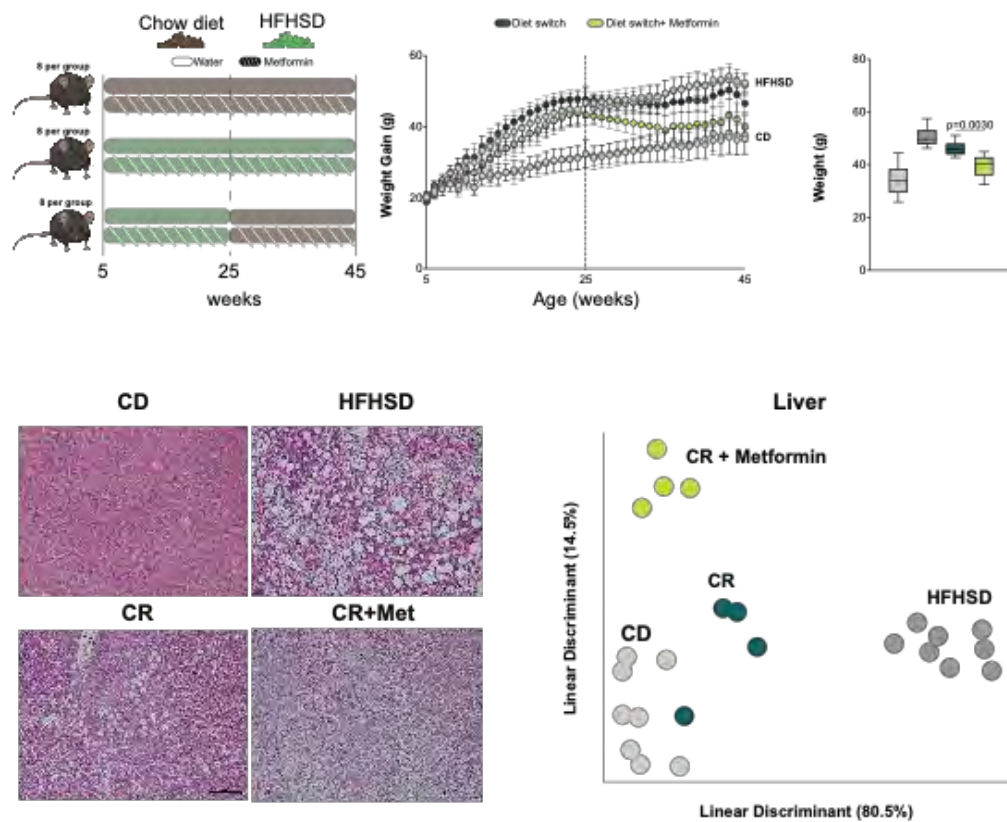
Considering that metformin can inhibit hepatic *de novo* lipogenesis, we hypothesized that the combination of CR and metformin can be more effective in ameliorating hepatic steatosis than CR alone.

Method: 5-week-old mice C57BL/6J were randomly allocated to receive a chow diet (CD, n = 8), CD with metformin (CD+Met, n = 8), high-fat high sucrose diet (HFHSD, n = 8), HFHSD with metformin (HFHSD+Met, n = 8) for 40 weeks of dietary treatment. After 20 weeks, two additional groups with HFHSD (n = 8) and HFHSD+Met (n = 8) 20 were switched to CD. After that, we performed biochemical, lipoprotein analysis from serum. We performed histological and lipidomics analyses from the liver as well as from visceral (epididymal and retroperitoneal), and subcutaneous (inguinal and anterior) adipocytes.

Results: Our results demonstrated that the synergies between calorie restriction and metformin reduce 19% of the body weight and induce hepatic steatosis remission in contrast to calorie restriction alone. The biochemical and lipoprotein concentration and profile were improved in both interventions, decreasing the glucose and LDL-p concentrations. The hepatic and adipocytes lipid signature improved with respect to HFHSD-fed mice, mimicking the signature of CD-fed mice. Thus, in the liver decreased the cholesterol esters and increased the bile acids and steroid hormones, PUFA-containing FA, NAE and LPC. The lipid remodelling in adipocytes from visceral and subcutaneous depots exhibited an increase in PUFA-containing DG, TG and LPC.

Conclusion: Herein, we demonstrate that CR combined with metformin induce NAFLD remission, whereas CR alone cannot.

Figure:



P07-18 Liraglutide ameliorates steatotic changes in a cell culture model of tamoxifen-induced steatosis by downregulation of lipogenic ACSL1 and SREBP-1c signaling pathways

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Background and aims: With the aging of the population, and increased prevalence of obesity, numerous global health issues emerge, such as polypharmacy with increased risk for drug-induced liver injury, and non-alcoholic fatty liver disease (NAFLD). These two entities may also co-exist, or drugs can worsen pre-existing NAFLD. The lack of appropriate diagnostic tools, understanding of the pathophysiological mechanisms, and adequate therapeutic solutions bring these diseases into the focus of various studies. Our study aimed to establish a cell culture model of tamoxifen-induced steatosis (TIS) and to assess the possible antisteatotic effect of liraglutide (glucagon-like peptide receptor 1 agonist) in it.

Method: Huh7 cells, a human liver cell line expressing the GLP-1 (glucagon-like peptide) receptor, were incubated with 2 M tamoxifen for 24 hours to establish the cell culture model of drug-induced hepatic steatosis. 5 nM to 20 nM liraglutide was also applied to the cells. Cell survival was determined using the erythrosin B staining exclusion assay. Oil-Red-O (ORO) and DAPI staining were used in fluorescence microscopy to measure changes in cell shape and the degree of hepatosteatois, respectively. TGO-PAP method was used to measure triglyceride content in cells. By using RT-PCR, the expression of different lipogenic genes and signals was evaluated.

Results: In the TIS model, tamoxifen significantly reduced cell survival ($p < 0,05$), while concurrent liraglutide treatment had no noticeable impact. ORO staining revealed a 5-fold increase in lipid accumulation in the TIS model ($p < 0,001$), which was caused by the significant rise in lipid droplet density. Liraglutide, however, reversed this impact and significantly reduced the number of lipid droplets as shown in Figure 1 ($p < 0,05$). Accordingly, triglyceride content was increased in TIS ($p < 0,05$), whereas liraglutide ameliorated it ($p < 0,001$). The lipogenic *ACSL1* and *SREBP-1c* gene expression signaling pathways, which were elevated in the TIS model, were significantly downregulated by liraglutide co-treatment ($p < 0,001$) (Figure 2).

Conclusion: Liraglutide's antisteatotic effects have been proven in the TIS cell culture model. Reducing the gene expression of several lipid synthesis-related factors, such as *ACSL1* and *SREBP-1c*, results in these effects.

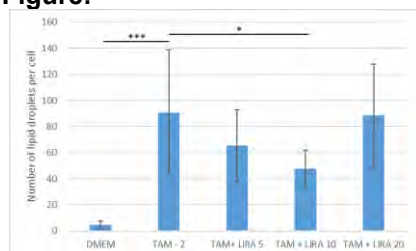
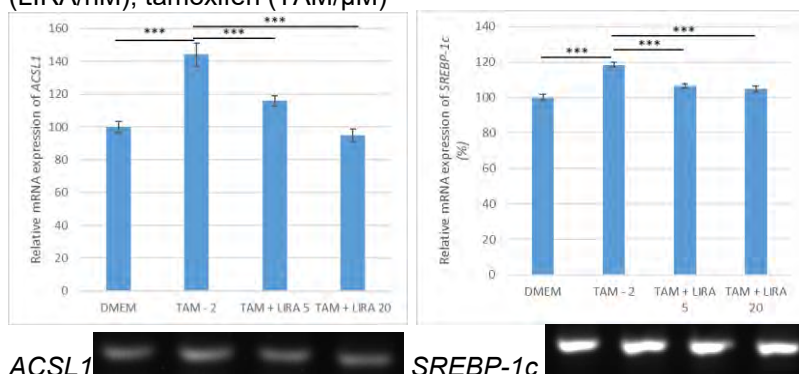
Figure:

Figure 1. Number of lipids droplets per cell after analyzing images of ORO stained Huh7 cells treated with TAM and LIRA. (* $p < 0.05$, *** $p < 0.001$); Dulbecco's Modified Eagle's Medium (DMEM), liraglutide (LIRA/nM), tamoxifen (TAM/ μ M)



ACSL1

SREBP-1c

Figure 2. Expression of *ACSL1* and *SREBP-1c* genes in cell culture models of TIS (***) $p < 0.001$); Dulbecco's Modified Eagle's Medium (DMEM), liraglutide (LIRA/nM), tamoxifen (TAM/ μ M)

P07-19 Aging contributes to the loss of glycerophospholipid in adipocytes in NAFLD mice model

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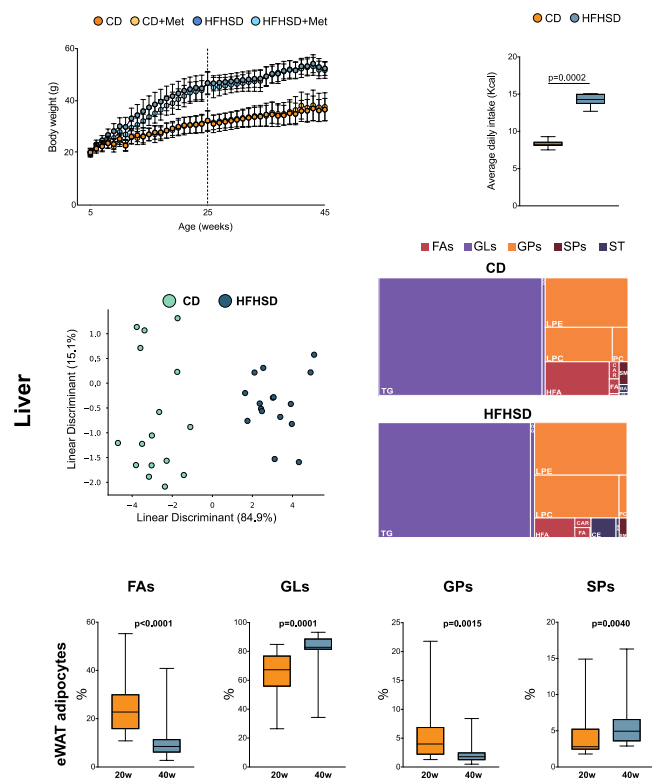
Background and aims: The metabolic reprogramming involved in the aging process is characterized by a transition from lipid mobilization to lipid storage, promoting lipid remodeling in metabolic key organs. Considering, that lipid accumulation is a hallmark of non-alcoholic fatty liver disease (NAFLD) we hypothesized that the lipid signature of this disease may be different according to age.

Method: 5-week-old mice C57BL/6J were randomly allocated to receive a chow diet (CD, n = 16), CD with metformin (CD+Met, n = 16), high-fat high sucrose diet (HFHSD, n = 16) and HFHSD with metformin (HFHSD+Met, n = 16) for 20 or 40 weeks of dietary treatment. After that, we performed histological analysis and lipidomics from the liver as well as from visceral (epididymal and retroperitoneal), and subcutaneous (inguinal and anterior) adipocytes.

Results: We observed that the hepatic lipid signature of HFHSD-fed mice was characterized by the increase in cholesterol esters (CE), carnitines (CAR), and fatty acids (FA), whereas the hepatic phosphatidylcholines (PC), bile acids (BAs) and steroid hormones (St) decreased compared to CD-fed mice and were age-independent. However, the adipocyte lipid signature changed with age. Thus, the lipid fingerprint in 45-week-old mice exhibited a loss of glycerophospholipids and an increase in glycolipids as well as the increase in SFA-containing CAR and PUFA containing-LPE and NAE species. Metformin cannot prevent NAFLD and age-associated metabolic effects.

Conclusion: Herein, we found that hepatic lipid signature was not age-dependent, and the findings were promoted by the HFHSD. However, the adipocyte lipid signature changed with age. These results invite us to consider whether it is necessary to develop a new treatment approach in NAFLD according to the age.

Figure:



P07-25 Hepatic senescence is associated with clinical progression of NAFLD/NASH: role of BMP4 and its antagonist Gremlin1

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Background and aims: Cellular senescence has attracted great attention as a potential contributor to the development of metabolic diseases and of NAFLD and NASH/cirrhosis. However, if it is just a marker or potential mediator of disease remains unclear. Moreover, key factors involved in regulating hepatocyte senescence in human NAFLD/NASH are largely unknown. Bone Morphogenetic Protein 4 (BMP4), a member of the TGF- β superfamily, has been shown to prevent hepatic steatosis in high fat diet (HFD)-fed mice but its potential role in the development and progression of NAFLD/NASH is not yet known.

Method: We performed extensive phenotyping and molecular characterization of 58 individuals with/without NAFLD/NASH. Further, we used an unbiased Machine Learning approach to identify key determinants of senescence. Moreover, the role of BMP4 and its antagonist Gremlin 1 was studied in senescent hepatocytes (following doxorubicin as a model of stress-induced senescence), as well as in 3D spheroids made from human stellate and hepatocyte cells.

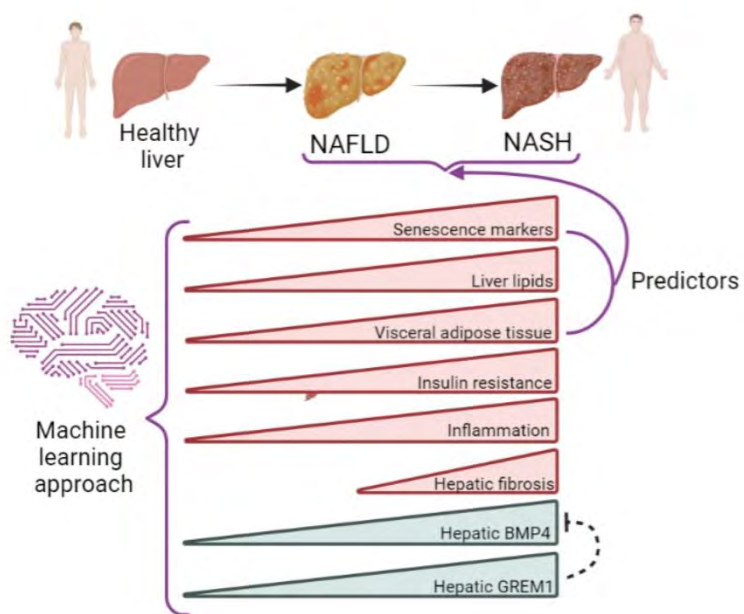
Results: Results showed that hepatic cell senescence is strongly related to NAFLD/NASH severity. Machine learning analysis identified senescence markers, the BMP4-inhibitor Gremlin 1 in liver and visceral fat as well as amount of visceral adipose tissue as strong predictors of NAFLD/NASH. Experimental studies in doxorubicin-induced senescent hepatocytes showed that BMP4 reduces senescence whereas Gremlin 1 promotes it. Using 3D model, we found BMP4 to be anti-senescent, anti-steatotic, anti-inflammatory and anti-fibrotic while Gremlin 1 is antagonistic to BMP4.

Conclusion: Our findings demonstrate that senescence is an important driver of human NAFLD/NASH and that BMP4 and Gremlin 1 are novel therapeutic targets.

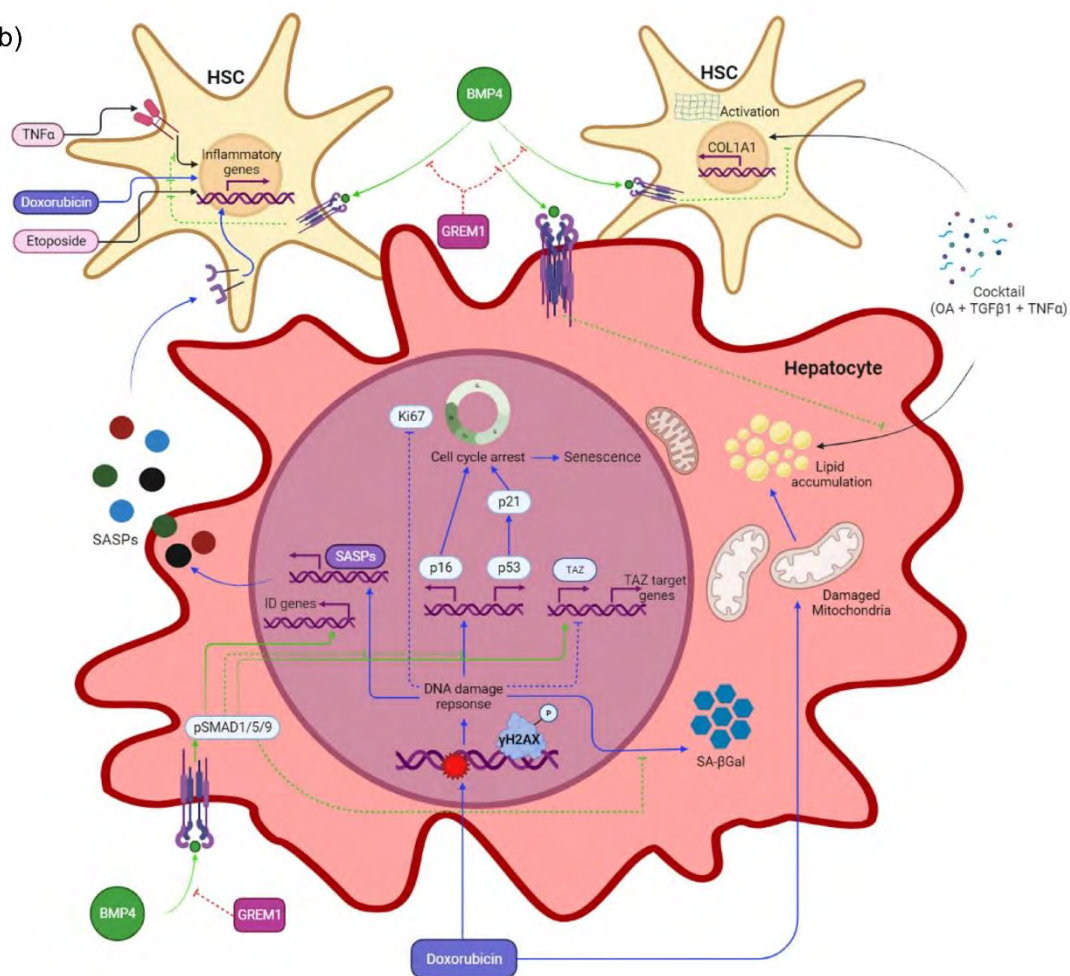
Figure:

Graphical abstract

a)



b)



**POSTER
ABSTRACT
PRESENTATION**

CLINICAL SCIENCE

P01-02 Pulse wave velocity is correlated with decline in renal filtration rate in non-alcoholic fatty liver disease patients

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Background and aims: Recent studies have suggested that arterial stiffness is a predictive factor for the decline of renal function in hypertensive and chronic renal failure patients, which increases their cardiovascular risk. The objective of our work was to estimate the relationship between carotidofemoral pulse wave velocity (cfPWV), pulse pressure (PP), and the presence of atherosclerotic plaques and GFR in non-alcoholic fatty liver disease (NAFLD).

Method: This is a prospective study that included non-diabetic patients over the age of 30. The diagnosis of steatosis and the search for carotid atheroma plaque (CAP) was made by ultrasound. Serum creatinine was measured using the compensated kinetic Jaffé method and glomerular filtration rate (GFR_e) was estimated using the CKD EPI KDIGO 2012 formula. Measurement of carotidofemoral pulse wave velocity (cfPWV) by Sphygmocor®. Statistical analysis: SPSS 25.0

Results: Two hundred and thirteen participants aged 48.52 ± 10.1 years including 113 women (53.1%) were included. The average PA was 132/78 mmHg. 32.8% were on antihypertensive treatment. Average estimated glomerular filtration rate (GFR_e) was 94.7 ± 16.5 ml/min $\times 1.73$ m², PP was 48.1 ± 14.1 mmHg and mean cfPWV was 10.7 ± 3.02 m/s. We noted a bivariate correlation between GFR_e, cfPWV ($r = -0.18$, $p = 0.007$), and the presence of CAP ($r = -0.2$, $p = 0.002$), but not in multivariate analysis.

Conclusion: Our results suggest that cfPWV and the presence of carotid atherosclerotic plaques may be markers of renal function decline in NAFLD patients.

P01-03 Sodium-glucose cotransporter 2 inhibitor versus sulfonylurea in patients with type 2 diabetes and non-alcoholic fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a liver phenotype of type 2 diabetes and obesity. To date, some anti-diabetic agents have been tested in participants with NAFLD. Currently, the efficacy of sodium-glucose cotransporter 2 (SGLT2) inhibitors and sulfonylureas in liver pathology and metabolic markers for type 2 diabetes with NAFLD are unknown.

Method: We conducted a 48-week, randomized, open-label, parallel-group trial involving participants with biopsy-confirmed NAFLD (NCT02649465). A total of 40 participants were randomly assigned to receive once-daily 20 mg tofogliflozin or 0.5 mg glimepiride. The primary outcome was the percentage of participants with at least an improvement in all individual scores for histological categories by at least one point.

Results: The participants were all Japanese and had type 2 diabetes. The mean age was 53.9 years, NAS 4.45, HbA1c 8.2%, and weight 82.0 kg. A total of 18 participants (45.0%) had stage F1 fibrosis, 11 (27.5%) had stage F2, and 5 (12.5%) had stage F3. Fibrosis scores improved in the tofogliflozin group (60%), whereas the change did not differ between the groups. All histologic variables: steatosis (65%), hepatocellular ballooning (55%), and lobular inflammation (50%) were improved in the tofogliflozin group, whereas only hepatocellular ballooning was improved in the glimepiride group (25%). The decrease in FPG and HbA1c were similar. Weight, BMI, and percentage of body fat were significantly reduced in the tofogliflozin group (a mean weight decrease of 4.2 kg). NAS improved significantly compared with baseline values in both groups, and the beneficial effects were greater in the tofogliflozin group ($p = 0.002$). There was an early and highly significant decrease in ALT and AST levels in the tofogliflozin group. The changes of γ -GTP were significantly reduced in the tofogliflozin group ($p < 0.001$ for the comparison with glimepiride). Moreover, the FIB-4 index was significantly reduced in the tofogliflozin group, and the effects were greater in the tofogliflozin group ($p = 0.015$).

Conclusion: Among participants with biopsy-confirmed NAFLD and type 2 diabetes, tofogliflozin administration was associated with a significant liver histology improvement compared with glimepiride under similar glucose level reduction. SGLT2 inhibitors may have a hepatoprotective effect, in addition to the previously recognized cardio-renal protective effects, and could be promising agents in the treatment of type 2 diabetes with NAFLD.

Figure: Hepatic histologic scores

Histologic Features	Tofogliflozin (N=20)			Glimepiride (N=20)			P value (Tofogliflozin vs. Glimepiride)‡
	Before	After	P Value†	Before	After	P Value†	
Steatosis							
Score-no. of subjects							
0(<5%)	0	5		0	0		
1(5-33%)	8	11		6	11		
2(33-66%)	8	3		9	5		
3(>66%)	4	1		5	4		
Improvement-%		65	0.001		30	0.058	0.141
Hepatocellular ballooning							
Score-no. of subjects							
0(None)	3	10		1	5		
1(Few balloon cells)	10	9		14	11		
2(Many balloon cells)	7	1		5	4		
Improvement-%		55	0.002		25	0.025	0.098
Lobular inflammation							
Score-no. of subjects							
0(0 focus)	1	4		0	0		
1(<2 foci per 200*field)	11	16		13	14		
2(2-4 foci per 200*field)	7	0		7	6		
3(>4 foci per 200*field)	1	0		0	0		
Improvement-%		50	0.003		15	0.655	0.064
Fibrosis							
Score-no. of subjects							
0(None)	3	10		2	6		
1(Perisinusoidal or periportal)	7	7		11	7		
2(Perisinusoidal and portal or periportal)	8	1		3	3		
3(Bridging fibrosis)	2	2		3	4		
4(Cirrhosis)	0	0		1	0		
Improvement-%		60	0.001		35	0.096	0.172

†The P values were calculated with the Wilcoxon signed-rank test. ‡ The between-group comparison for the effect of treatment (change from baseline) was performed with the χ^2 test

P01-06 Contribution of "multiple hit " to NAFLD pathology differ in obese and non obese population

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Background and aims: Non-Alcoholic fatty liver disease (NAFLD) can exist in both obese and non-obese individuals. There is considerable debate regarding whether the pathogenesis differs among the subsets. Keeping this in mind we tried to investigate the progression of disease among obese and non-obese individual, along with the role of systemic inflammation and gut permeability in the pathogenesis of NAFLD.

Method: 55 biopsy proven NAFLD subjects, consisting both obese and lean individuals were assessed. Biochemical (Fasting Plasma Glucose, Glycated Hemoglobin, Lipid Profile, Liver Function tests) and histological parameters (NAS Score and SAF Score) were compared. C-Reactive Protein (CRP) and D-Lactate was measured as markers of systemic inflammation and gut permeability respectively. HOMA modelling was done to calculate Insulin Resistance and Insulin Secretion.

Descriptive summary of the data was represented by mean and standard deviation. 95% confidence interval has been presented where relevant. Shapiro-Wilk's W test was performed to assess normality. Numerical variables were compared between groups by independent-samples t-test or Man-Whitney U test as appropriate. Categorical variables were compared using Chi-square test. Patients were divided into 2 groups- Nonobese (BMI <25) and Obese (BMI ≥25) and comparative analysis were done between these 2 groups. Data analysis was performed in RStudio (Version 1.1.447).

Results: Parameters of insulin resistance and secretion, liver function test, lipid profile and glycemic parameters were comparable between the groups. Interestingly, the NAS score was also comparable, with only the fibrosis component in the SAF score significantly increased in the obese BMI group. Moreover, CRP and D-lactate (bacterial metabolite) both were reduced in the non-obese group. A comparative analysis of CRP and D-Lactate across different fibrosis scores (0, 1 and 2) reveals that both CRP and D-Lactate levels increase with increasing fibrosis scores.

Conclusion: Reduced fibrosis among non-obese NAFLD patients despite of comparable disease activity suggests that the disease progresses in a different manner among non-obese individuals. Moreover, association of CRP and D-Lactate with fibrosis score reveal that systemic inflammation and gut permeability mayn't be that important in NAFLD pathology in the non-obese group. Considering the "multiple-hit" hypothesis we may, hereby, hypothesize that the contribution of multiple hits to NAFLD pathology differ in non-obese and obese NAFLD groups.

Figure:

TABLE-1:

Clinico-pathological characteristics of NAFLD patients			
Variables	Non-obese (BMI < 25)	Obese (BMI ≥ 25)	P value
N	13	42	
Sex (Male/Female)	8/5	19/23	0.478
Age (years)	38.31 ± 2.97	40.14 ± 1.37	0.302
Fasting Plasma Glucose (mg/dL)	90 ± 3.23	104.81 ± 6.63	0.378
Glycated Haemoglobin (%)	5.55 ± 0.15	6.25 ± 0.23	0.095
HOMA-IR	3.19 ± 0.98	3.55 ± 0.41	0.416
HOMA-B	183.8 ± 46.21	190.62 ± 26.52	0.977
Liver Function Tests			
Total Bilirubin (mg/dL)	0.94 ± 0.12	0.98 ± 0.08	0.764
Conjugated Bilirubin (mg/dL)	0.3 ± 0.04	0.31 ± 0.03	0.536
Serum Albumin (g/dL)	4.65 ± 0.08	4.75 ± 0.08	0.668
Serum Globulin (g/dL)	2.89 ± 0.14	3.12 ± 0.08	0.18
Alanine Aminotransferase (IU/L)	46.85 ± 6.17	63.38 ± 8.43	0.446
Aspartate Aminotransferase (IU/L)	39.54 ± 5.17	50.38 ± 5.75	0.416
Alkaline Phosphatase (IU/L)	141.54 ± 13.58	150.76 ± 6.69	0.677
Gamma-glutamyltransferase (IU/L)	41.08 ± 4.86	56.9 ± 6.21	0.271
Lipid Profile			
Total Cholesterol (mg/dL)	193.69 ± 15.51	195.69 ± 6.9	0.895
HDL-Cholesterol (mg/dL)	38 ± 1.22	40.57 ± 0.94	0.164
LDL-Cholesterol (mg/dL)	117.62 ± 14.89	121.05 ± 5.64	0.793
VLDL-Cholesterol (mg/dL)	32.23 ± 3.43	35.19 ± 2.11	0.592
Triglycerides (mg/dL)	174.31 ± 25.45	172.14 ± 8.59	0.677
Inflammation Markers			
C-Reactive Protein (mg/dL)	5.72 ± 2.01	8.71 ± 1.35	0.048
Plasma D-Lactate (μM)	54.48 ± 10.22	84.41 ± 8.92	0.008
NASH CRN Score			
NAS Score	1.85 ± 0.54	2.55 ± 0.25	0.175
Components of SAF Score			
Steatosis	1.23 ± 0.32	1.52 ± 0.13	0.369
Activity	0.62 ± 0.24	0.98 ± 0.15	0.233
Fibrosis	0.38 ± 0.21	1 ± 0.16	0.035

Comparison of clinical and histological parameters between non-obese and obese NAFLD patients. Data represented as mean ± standard error of the mean, p value < 0.05 considered to be significant.

P01-07 Association between advanced stage of liver fibrosis in patients with metabolic fatty liver disease and intestinal permeability

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Background and aims: nowadays, metabolic fatty liver disease (MAFLD) is widely spread through the world. Besides, liver fibrosis is considered as a universal factor for the patient survival. It is considered that the assessment of intestinal permeability is a promising objective for the study and selection of therapy.

We want to detect the clinical and laboratory markers associated with the advanced stages of fibrosis, including of the assessment intestinal permeability and adipokines, in patients with MAFLD.

Method: 216 patients with MAFLD (133 men (61.6%) and 84 women (38.9%), average age 53 years) were included into the open comparative study. A standard laboratory and instrumental examination, including elastography to assess the stage of liver fibrosis was carried out. Furthermore, serum concentrations of insulin, leptin, its soluble receptor, adiponectin and zonulin in stool were determined. The diagnostic value of the parameters obtained by the comparative and correlation methods were studied in the groups of patients with MAFLD depends on stage of liver fibrosis (F0-3).

Results: All patients were obese or overweight (average BMI 33.5 kg/m²) and had a higher waist volume than hip volume (ratio WV/HV 1.1). Positive correlations between the stage of fibrosis and abdominal obesity were found ($r_s = 0.510$, $p \leq 0.01$). At the time due to examination, 104 patients with NASH were identified and the FIB-4 index averaged 1.29 ± 0.8 . According to elastometry data, 34 patients with advanced stages of fibrosis (F2-3) were identified. Patients with advanced stages of fibrosis had a higher level the HOMA-IR index (T-criterion = -1.682 , $p \leq 0.01$), the insulin (T-criterion = -1.364 , $p \leq 0.01$) and a lower level of adiponectin (T-criterion = 0.988 , $p \leq 0.01$). The level of zonulin in stool was significantly higher in patients with significant fibrosis (T-criterion = -1.293 , $p \leq 0.01$). A positive correlation between the level of zonulin in stool was revealed.

Conclusion: liver fibrosis in patients with MAFLD is associated with abdominal obesity, insulin resistance and low adiponectin levels. Patients with MAFLD had features of increased intestinal permeability, detecting by zonulin level in stool. Zonulin in stool is a promising non-invasive marker of intestinal permeability in patients with MAFLD.

P01-09-YI Predictors of steatohepatitis in hypertensive non-alcoholic fatty liver disease patients

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Background and aims: High prevalence of non-alcoholic fatty liver disease (NAFLD) is broad socio-economical problem in healthcare. Numerous associations of NAFLD and hypertension (HTN) are found. Selenoprotein P (Sel P), which contains Selenium (Sel), is the glycoprotein, which regulates antioxidant, endocrine and immune function. This depicts the need in research of Selenium role in NAFLD development. Aim was to investigate the predictors of steatohepatitis in hypertensive NAFLD patients.

Method: Study included 100 NAFLD patients. Main group: 49 hypertensive NAFLD patients (67.3 % females); comparison group: 51 nonhypertensive NAFLD patients (58.8 % females). Control group: 20 individuals (55.0 % females). Groups were gender-matched ($p = 0, 544$).

Respective median age was 51.0 [45.0-56.0] years, 52.0 [47.0-54.0] years and 51.0 [45.0-55.5] years. Groups were age-matched ($p_{1-2} = 0, 610$, $p_{1-3} = 0, 980$, $p_{2-3} = 0, 544$).

In main group 27 (55.1 %) of patients had steatosis and 22 (44.9 %) had steatohepatitis. In comparison group: 30 (58.8 %) and 21 (41.2 %) respectively ($p_{1-2} = 0, 707$).

Aspartate aminotransferase (AST) was measured by kinetic method; Sel P and Sel — by respective ELISA kits.

Results: Baseline characteristics are shown in table. In steatohepatitis patients SBP was significantly ($p \leq 0.001$) higher than in steatosis. Steatohepatitis patients showed significantly ($p < 0.001$) higher ASL levels. Levels of Sel P and Sel were higher in steatohepatitis but were not statistically significant.

Multivariate regression analysis revealed direct association of levels of AST (OR = 1, 421 [95, 0 % CI 1, 198-1, 687], $p < 0, 001$), Sel P (OR = 1, 143 [95, 0 % CI 1, 068-1, 224], $p < 0, 001$), Sel (OR = 1, 054 [95, 0 % CI 1, 012-1, 098], $p = 0, 011$) and SBP (OR = 1, 089 [95, 0 % CI 1, 017-1, 116], $p = 0, 014$) with steatohepatitis development in NAFLD patients.

Conclusion: Steatohepatitis predictors in NAFLD patients were Selenium, Selenoprotein P, AST and SBP. Findings indicate the negative effect of increased systemic blood pressure on functional state of liver, and demonstrate possible therapeutic potential of Selenium usage in hypertensive NAFLD patients.

Figure:

Baseline parameters in studied patients, Me [Lq; Uq]

Parameter		Steatosis (n = 57)	Steatohepatitis (n = 43)	p
<i>SBP, mm Hg.</i>	<i>Total</i>	130.0 [115.0-145.0]	145.0 [130.0-155.0]	0, 001
	<i>NAFLD+HTN</i>	145.0 [140.0-150.0]	155.0 [150.0-160.0]	<0, 001
	<i>NAFLD</i>	117.5 [110.0-125.0]	130.0 [127.5-135.0]	<0, 001
<i>AST, U/L</i>	<i>Total</i>	43.0 [40.0-51.0]	50.0 [45.0-57.0]	<0, 001
	<i>NAFLD+HTN</i>	51.0 [49.0-53.0]	56.5 [54.0-57.0]	<0, 001
	<i>NAFLD</i>	40.0 [39.0-41.0]	45.0 [43.0-48.0]	<0, 001
<i>Selenium, mkg/L</i>	<i>Total</i>	56.9 [42.4-71.6]	54.6 [44.4-67.2]	0, 826
	<i>NAFLD+HTN</i>	42.4 [34.5-39.5]	46.0 [42.4-49.5]	0, 169
	<i>NAFLD</i>	69.9 [62.4-77.5]	66.4 [57.0-78.1]	0, 394
<i>SeI P, ng/ml</i>	<i>Total</i>	39.6 [21.3-43.5]	36.1 [19.2-43.2]	0, 870
	<i>NAFLD+HTN</i>	19.9 [7.3-26.7]	19.5 [8.0-26.8]	0, 817
	<i>NAFLD</i>	42.7 [40.7-45.5]	43.2 [42.2-46.0]	0, 559

P01-10 Relationship between endothelial dysfunction and non-alcoholic fatty liver disease

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Background and aims: It is currently considered that non-alcoholic fatty liver disease (NAFLD) is a risk factor for cardiovascular disease. The presence of cardiovascular complications of NAFLD worsens its course and prognosis for patients. In recent years, researchers have focused on the features of intercellular interaction and endothelial dysfunction (ED) as a factor in vascular damage in patients with NAFLD, but endothelial dysfunction has not been studied enough.

The aim-is to evaluate the relationship between markers of systemic inflammation, degree of liver fibrosis and endothelial dysfunction in patients with NAFLD.

Method: We examined 241 patients and determined the level of inflammatory mediators, endothelin (ET-1), the activity of the Willebrand's factor (vWF), the thickness of the intima-media complex, presence of atherosclerotic plaque and stenosis of the carotid arteries, index HOMA-IR. The ratio between the content of adiponectin and leptin was represented as log A/L. The anthropometric survey, measured levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), the degree of liver fibrosis using elastography (FibroScan), ECG and echocardiography were conducted

Results: Correlation analysis revealed a direct correlation between HOMA-IR and leptin ($r = 0.8$; $p = 0.00166$) and inverse correlation between HOMA-IR and adiponectin ($r = -0.66$; $p = 0.0033$) and index log A/L ($r = -0.71$; $p = 0.0000$) It is evident that the decrease in the concentration of adiponectin with a parallel increase in the content of leptin increases IP. A comparative analysis of the level of CRP inflammation marker in obese patients showed a direct relationship with HOMA-IR ($r = 0.58$; $p = 0.05$), glucose ($r = 0.44$; $p = 0.0045$) and insulin ($r = 0.66$; $p = 0.0001$) in the blood. The patients with NAFLD by obesity showed a reduction in endothelium-dependent vasodilation, indicating the presence of endothelial dysfunction. The concentration of proinflammatory cytokines such as TNF- α and IL-6 in patients with NAFLD was 3-7 times higher than the similar parameters of patients with a similar degree of obesity but without evidence NAFLD. The concentration of ET-1 in the blood plasma of patients with NAFLD has a strong direct correlation with the degree of cardiovascular risk and cognitive deficit in surveyed patients. It is found that many inflammatory mediators (TNF- α , IL-1, IL-6) and markers (C-reactive protein, fibrinogen) highly correlate with the degree of obesity, the concentration of ET-1, vWF and markers of insulin resistance a predictor of cardiovascular risk.

Conclusion: The development of NAFLD is associated with the development of endothelial dysfunction, increasing levels of leptin and markers of systemic inflammation, decreasing levels of adiponectin in patients with NAFLD.

P01-11-YI Liver stiffness is associated with excess mortality in the general population driven by heart failure: The Rotterdam study

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Background and aims: Elevated liver stiffness may reflect hepatic fibrosis but can also be secondary to venous congestion. We aimed to study the association between liver stiffness and mortality in the general population, stratified for heart failure (HF) and/or coronary heart disease (CHD).

Method: We analyzed individuals enrolled in the ongoing prospective population-based Rotterdam Study who attended a visit between 2009 and 2014 with available liver stiffness measurement. Exclusion criteria were alcohol abuse, viral hepatitis, incomplete data on HF and unreliable liver stiffness measurements. The association between liver stiffness and mortality was assessed in the overall population and after stratification by HF/CHD, using Cox regression. Final models were adjusted for age, sex, smoking, alcohol, steatosis and the individual components of the metabolic syndrome. Additionally, associations between HF, CHD, and echocardiographic characteristics and liver stiffness were quantified with linear regression.

Results: The 4,153 included participants were 67.5 ± 8.4 years, 44.2% was male and metabolic comorbidity was highly prevalent (46.7% metabolic syndrome, 13.8% diabetes). The median liver stiffness was 4.8 kPa [3.9-5.9] and exceeded 8.0 kPa in 6.2%. During the median follow-up of 6.0 [5.1-7.0] years, 373 deaths were recorded, resulting in a mortality rate of 15.1 per 1,000 person-years. In the overall population, liver stiffness ≥ 8.0 kPa was associated with excess mortality (adjusted hazard ratio [aHR] 1.37, 95%CI 1.00-1.89). However, this association was entirely driven by participants with heart failure (aHR 2.48, 95%CI 1.15-5.35), whereas no association was observed between liver stiffness and mortality in subjects without HF and/or CHD (aHR 1.07, 95%CI 0.70-1.64). Several cardiovascular characteristics were significantly associated with higher liver stiffness, including a previous diagnosis of HF, moderate to poor diastolic dysfunction, and right atrium diameter over 4.5 cm (effects ranging from +0.7 to +1.9 kPa, $p < 0.05$).

Conclusion: In this large population-based study, we demonstrated that high liver stiffness was associated with excess mortality, but this result was entirely driven by HF. Furthermore, a range of cardiovascular characteristics and heart failure were associated with an increase in liver stiffness. These findings highlight important limitations of elastography-based screening for advanced liver disease in low-prevalence populations.

P01-14 Sex-specific hepatoprotective effects of long-term semaglutide treatment in GAN diet-induced obese and biopsy-confirmed mouse model of NASH

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Background and aims: The glucagon-like-peptide (GLP)-1 analogue semaglutide is in advanced clinical development for non-alcoholic steatohepatitis (NASH). The present study aimed to compare sex-specific metabolic and hepatic efficacy profiles of long-term semaglutide treatment in the GAN (Gubra-Amylin NASH) diet-induced obese (DIO) mouse model of NASH.

Method: Male and female C57BL/6J mice were fed chow or the GAN diet high in fat, fructose, and cholesterol for 38 weeks prior to study start. Only DIO-NASH animals with liver biopsy-confirmed NAFLD Activity Score (NAS ≥ 4) and fibrosis (stage $\geq F1$) were included and stratified into treatment groups. DIO-NASH mice (n = 16-18) received vehicle, semaglutide (30 nmol/kg, SC, QD), and Chow animals (n = 10) received vehicle, for total of 24 weeks. Pre-to-post liver biopsy histology was performed for within-subject evaluation of NAS and Fibrosis Stage. Additional end points included blood and liver biochemistry and quantitative liver histology.

Results: Compared to respective chow mice, vehicle-treated male and female DIO-NASH mice developed metabolic, biochemical, and histological hallmarks of NASH. Notably, disease progression was more pronounced in male DIO-NASH mice with consistent development of advanced disease activity (NAS ≥ 5) and fibrosis (stage F3; bridging fibrosis). Semaglutide promoted marked weight loss ($\approx 20\%$) with significant improvements in hepatomegaly, liver lipids and plasma transaminases in both male and female DIO-NASH mice as compared to corresponding vehicle controls. In male DIO-NASH mice, semaglutide significantly improved NAS by ≥ 2 point, driven by reduction in steatosis and lobular inflammation scores. In contrast, semaglutide induced anti-steatotic action in female DIO-NASH mice, albeit without improving NAS. Histopathological scores were supported by reductions in histomorphometric markers of steatosis and inflammation. Semaglutide did not improve fibrosis stage in neither male nor female DIO-NASH mice, albeit male DIO-NASH mice demonstrated significantly reduced levels of collagen 1a1 and α -SMA levels (marker of stellate cell activation), indicating efficacy on fibrogenesis.

Conclusion: Sex-specific disease progression is being recapitulated in GAN DIO-NASH mice, highlighting clinical translatability. While long-term semaglutide treatment promoted robust metabolic therapeutic effects in males and females, sex-specific benefits on histopathological hallmarks of NASH were clearly observed in male GAN DIO-NASH mice. This could potentially suggest gender differences in the response to semaglutide, arguing for preclinical drug efficacy profiling in both male and female GAN DIO-NASH mice.

P01-15 Meat consumption and PNPLA3 polymorphism are associated with fatty liver in a multi-center cross-sectional study

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Background and aims: One single-nucleotide polymorphism (SNPs) rs738409 in the patatin-like phospholipase domain-containing 3 gene (PNPLA3) has been implicated in susceptibility to NAFLD and liver fibrosis. Recent studies demonstrate an association between red and processed meat consumption and NAFLD. However, the synergistic effect between meat and genetic polymorphism with NAFLD has not been thoroughly tested. The current study aimed to test the association between PNPLA3, meat consumption, and their plausible interaction with NAFLD and liver-related damage.

Method: A cross-sectional study among three outpatient samples in three medical centers in 1) population undergoing screening colonoscopy at the Tel-Aviv medical center, Israel (n = 221). 2) primary health care patients from Porto Alegre, Brazil (n = 299). 3) Biopsy-proven NAFLD patients under medical follow-up in a Tertiary Center from Sao Paulo, Brazil (n = 87). Data about physical activity, smoking habits, and alcohol consumption were collected by questionnaires. Food consumption was assessed by a food frequency questionnaire (FFQ). High meat consumption was considered as consumption above the specific center median. Genomic DNA was extracted from whole blood samples and analyzed by TaqMan Real Time-PCR. PNPLA3 polymorphism was defined as homozygous (GG) or heterozygous (GC), while homozygous (CC) for the rs738409 PNPLA3 C allele was classified as no polymorphism. Fatty liver (FL) was defined as FL index (FLI) ≥ 30. "Significant liver disease" was defined by histologic findings of three and above features (steatosis, fibrosis, lobular inflammation, or ballooning ≥ 2).

Results: 607 subjects (36.70% male, mean age 61.40 ± 11.45 years, mean BMI (28.83 ± 5.12 Kg/m²) was included. According to FLI, the pooled prevalence of FL in the screening and primary care populations was 62.40%, and PNPLA3 polymorphism was 52.00%. There was a significant association between high red and/or processed meat, unprocessed red meat, and processed meat with FL only among subjects with PNPLA3 polymorphism (OR = 2.47, 95%CI 1.02-5.96, P = 0.044; OR = 2.61, 1.08-6.35, P = 0.034; OR = 2.63, 1.14-6.09, P = 0.024, respectively), in a multivariable analysis, adjusting for nutritional and lifestyle habits. Among the biopsy proven NAFLD sample, subjects with both PNPLA3 polymorphism and with high consumption of red and/or processed meat or unprocessed red meat were with the highest odds for "significant liver disease" compared to subjects with any of the risk factors (OR = 4.78, 95% CI 1.02-22.41, P = 0.047; OR = 5.73, 1.19-27.63, P = 0.030, respectively) adjusted for age, gender, BMI and saturate fatty acids.

Conclusion: High meat intake and PNPLA3 polymorphism have a synergistic effect on NAFLD and liver fibrosis in heterogeneous populations. If confirmed, it may be recommended for people with polymorphism to avoid high red meat consumption.

P01-16 Primary data analyses of MAESTRO-NAFLD-1: a 52-week randomized double-blind placebo-controlled Phase 3 trial of resmetirom in patients with non-alcoholic fatty liver disease

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Background and aims: MAESTRO-NAFLD-1 (NCT04197479) is a randomized double-blind placebo-controlled Phase 3 trial evaluating the safety of resmetirom (MGL-3196), a liver-targeted, oral, once-daily thyroid hormone receptor-beta selective agonist, in >1100 patients with non-alcoholic fatty liver disease (NAFLD).

Method: Enrollment occurred between December 2019 and October 2020 at 79 US sites. Requirements included ≥ 3 metabolic risk factors, FibroScan VCTE ≥ 5.5 kPa/CAP ≥ 280 dBm, and MRI-PDFF $\geq 8\%$. Randomization was 1:1:1:1 to 3 double-blind arms (80 mg resmetirom, 100 mg resmetirom, or placebo [n = 972]) or an open-label arm (100 mg resmetirom [n = 171]). The primary objective was to evaluate the safety and tolerability of 80 or 100 mg resmetirom versus placebo as measured by the incidence of adverse events (AEs).

Results: At baseline, the double-blind safety population (n = 969) was 55.9 years (11.8); 54.4% were female; 88.6% were White; 34.7% were Hispanic or Latino; BMI 35.3 kg/m² (6.0); 49.0% had type 2 diabetes; 76.1% had hypertension; 87.9% had dyslipidemia; FibroScan VCTE 7.4 kPa (4.7). Discontinuations did not differ by treatment (22.5%); most were due to patient decision (COVID-19 related). Double-blind compliance was impacted by drug kit delays due to COVID-19. Withdrawals due to AEs occurred in 2.4% (80 mg), 2.8% (100 mg), and 1.3% (placebo) of patients. TEAEs occurred in 88.4% (80 mg), 86.1% (100 mg), and 81.8% (placebo) of patients. TEAEs \geq grade 3 severity were reported in 7.6% (80 mg), 9.0% (100 mg), and 9.1% (placebo) of patients. AEs in excess of placebo were grade 1-2 AEs of diarrhea (23.5% [80 mg]/31.2% [100 mg] vs 13.8% [placebo]) and nausea (11.9% [80 mg]/18.2% [100 mg] vs 7.9% [placebo]) in the first few weeks of resmetirom treatment. ALT increases $\geq 3\times$ ULN were observed in 0.61% (80 mg), 0.31% (100 mg), and 1.6% (placebo) of patients. There were no changes in body weight or heart rate. BP decreased by 2-3mmHg in the resmetirom arms. Key secondary end points were met (**Table**). Comparative mean reduction in FibroScan VCTE was not significant; responder analysis of FibroScan and MRE showed significant reductions with resmetirom treatment.

Conclusion: Resmetirom was well tolerated over 52 weeks in adults with NAFLD who were identified by metabolic risk and non-invasive imaging. Key secondary end points were met including LDL-C, apoB, triglycerides, MRI-PDFF, and FibroScan CAP.

Figure:

Table.							
	Resmetirom 100 mg OL (n=171)	P value	Resmetirom 100 mg DB (n=314)	P value	Resmetirom 80 mg DB (n=320)	P value	Placebo (n=309)
LDL-C, %CFB (SE) at Week 24	-21 (1.9)	<0.0001	-14.4 (2.1)	<0.0001	-12.7 (2.1)	<0.0001	-1.7 (2.0)
ApoB, %CFB (SE) at Week 24	-22 (1.5)	<0.0001	-16.6 (1.6)	<0.0001	-14.6 (1.5)	<0.0001	-0.1 (1.5)
MRI-PDFF, %CFB at Week 16	-48.9%	<0.0001	-47.7%	<0.0001	-40.8%	<0.0001	-6.0%
MRI-PDFF, %CFB at Week 52	-52.5%	<0.0001	-48.2%	<0.0001	-42.5%	<0.0001	-7.9%
Triglycerides, %CFB at Week 24 (BL ≥150 mg/dL)	-25 (3.1)	<0.0001	-21.5 (-28.0, -14.3)	<0.0001	-19.5 (-27.0, -11.1)	0.0005	-2.1 (-10.6, 7.4)
FibroScan CAP at Week 52	-52.9 (4.6)	<0.0001	-42.2 (4.1)	<0.0001	-36.2 (4.0)	<0.0001	-17.8
FibroScan VCTE at Week 52 (BL ≥7.2 kPa; ≥2 kPa reduction)	57.1%	0.0005	42.7%	0.020	31.9%	0.38	24.7%
MRE (BL ≥2.9 kPa; ≥19% reduction)	25.7%		22.6%	0.024*	21.7%		11.4%
ALT, %CFB (BL ≥30 IU/L)	-29.4 (3.3)	<0.0001	-17.9 (5.0)	0.0010	-18.5 (4.8)	0.0015	-2.1 (5.1)

*MRE combined resmetirom groups.
 ALT, alanine aminotransferase; apoB, apolipoprotein B; BL, baseline; CAP, controlled attenuation parameter; CFB, change from baseline; DB, double-blind;
 LDL-C, low-density lipoprotein cholesterol; MRE, magnetic resonance elastography; MRI-PDFF, magnetic resonance imaging-proton density fat fraction;
 OL, open-label; SE, standard error; VCTE, vibration-controlled transient elastography.

P01-17-YI Non-alcoholic fatty liver disease is associated to post-acute COVID syndrome

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Background and aims: Post-Acute COVID syndrome (PACS) is an increasingly widespread emerging nosological entity, affecting millions of people all over the world. The aim of our study is to assess the prevalence of steatosis in a cohort of patients previously hospitalized for COVID-19 and with PACS.

Method: We enrolled consecutive patients attending the post-acute outpatient service for individuals after recovery from COVID-19.

Patients were considered suffering of PACS in case of persistence of symptoms (present or not at the onset of the infection) after 4 weeks of infection, with a permanent, relapsing/remitting or progressive improvement course or/and in case irreversible tissue damage after 12 weeks that could trigger different degrees of permanent dysfunction and associated symptomatology.

The diagnosis of steatosis was performed according to ultrasound criteria or non-invasive fibrosis scores. Data was collected during in a single multispecialistic assessment visit at 121-day (SD \pm 89.2) from hospital discharge. We recorded symptoms or sequelae of COVID-19 acute infection and laboratory tests.

Results: Of 2092 patients, 301 were excluded due to lack of data or due to active cancer or other liver disease than NAFLD. Of the remaining, 1395 had PACS and 396 showed no PACS features.

Mean age was comparable between two groups (54.6 years; SD \pm 16.2 vs 54.6 SD \pm 13.9 p = 0.961). Women were more frequently affected by PACS (51.4%, p < 0, 001). Patients in the PACS group were more frequently overweight (BMI: 26.7 SD \pm 4.9 vs 25.4 SD \pm 4.0, p < 0.001), obese (21.4% vs 9.3%, p < 0, 001), more likely to be affected by hypertension (45.4% vs 39.4%, p = 0.032) and by hypertriglyceridemia (19.9% vs 14.1%, p < 0.001). Metabolic syndrome was more frequent in patients without PACS (83.6% vs 75.4%, p < 0.001). There were no significant differences regarding the other major comorbidities.

Concerning laboratory findings there were no differences except for triglycerides and albumin was slightly lower in the PACS group.

Remarkably, patients with PACS were more likely affected by steatosis (49.3% vs 27.3%, p < 0.001). Univariate analysis was performed and showed higher rates of PACS in patients with NAFLD (odds ratio [OR] 2.60, CI, 2.04-3.33, p < 0.001). Logistic regression analysis identified the presence of NAFLD and hypertriglyceridemia as independent predictors of PACS (OR: 2.27, CI, 1.69-3.08, p < 0.001 and OR 1.42, CI, 1.01-2.02, p = 0.050, respectively).

Conclusion: Our results clearly show that the presence of NAFLD represents an independent risk factor for the onset of PACS in a selected population of patients previously hospitalized for COVID-19.

P01-18 Clinical characteristics of non-alcoholic fatty liver disease in pregnant women with varying degrees of obesity

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Background and aims: Today, non-alcoholic fatty liver disease (NAFLD) is a leading problem among overweight pregnant women, as the incidence of obesity among women of childbearing age is on the rise. Non-compliance with the basics of nutrition leads to violation of lipid metabolism, which lead to the formation of obstetric and perinatal complications. Our aim is the evaluation the functional status of the liver in pregnant women with NAFLD depending on body mass index.

Method: We've examined 98 pregnant women with NAFLD at the stage of NASH in combination with obesity. The control group consisted of 30 almost healthy pregnant women. Depending on the body mass index (BMI), all surveyed women are divided into three groups: Group I-overweight, Group II-grade I obesity, Group III-grade II obesity. The main clinical and biochemical syndromes were evaluated.

Results: During the non-invasive diagnostics of the liver we revealed a tendency to increase the degree of steatosis in the examined groups depending on the increasing BMI. According to the results of the Steato-test, the highest rate was observed in group III, which was 1.38, 1.2 and 4.2 times higher than the results of the examined I and II groups and the control group ($p < 0.05$). NASH-test data among women with NASH on the background of grade II-III obesity exceeded 1.54 times the data obtained among women of group I, 1.11 times in group II and 4.5 times the results among control ($p < 0.05$). Comparing the clinical manifestations of NAFLD in pregnant, the highest frequency is observed in the group with severe obesity compared with the group of patients with moderate obesity and overweight: symptoms of asthenic syndrome in 91.6.0%, 79.1% and 61.5% of patients ($p < 0.05$), manifestations of dyspepsia-in 87.5%, 54% and 34.6% patients ($p < 0.05$), feeling of heaviness or moderate pain in the right hypochondrium-in 62.5% 50% and 30.7% of patients, respectively ($p < 0.05$).

Conclusion: It has been established pregnant women with NAFLD have pronounced clinical picture of the disease. Liver dysfunction occurs on the background of grade I obesity, which can be considered as an early marker of steatohepatitis.

Figure:

Table 1. Incidence of major clinical and biochemical syndromes of steatohepatitis in pregnant women with overweight, with NASH and obesity I degree and with NASH with obesity II-III degree

Syndroms	Overweight I group (n = 26)		Obesity: class I II group (n = 48)		Obesity: class II-III group III (n = 24)	
	Abs.	%	Abs.	%	Abs.	%
Asthenic	16	61, 5	38	79, 1	22	91, 6
Dyspeptic	9	34, 6	26	54, 1	21	87, 5
Dyskomfort/pain in the right hypochondrium	8	30, 7	24	50, 0	15	62, 5
Hepatomegaly	5	19, 2	30	62, 5	19	79, 1
Cytolytic	3	11, 5	37	77, 0	21	87, 5
Cholestatic	4	15, 3	6	12, 5	4	16, 6
Mesenchymal- inflammatory	8	30, 7	18	37, 5	14	58, 3
Hepatocellular insufficiency	2	7, 6	12	25, 0	12	50, 0
Impaired glucose tolerance	11	42, 3	33	68, 75	19	79, 1
Hyper-, dyslipidemia	14	53, 8	39	81, 0	50	100, 0

P01-19-YI Validation of elastography criteria and cACLD risk model for diagnosis of compensated advanced Chronic liver disease (cACLD) in a UK cohort of NAFLD patients

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Background and aims: Fibroscan is a well-established NIT for the diagnosis of advanced fibrosis (F>2) in patients with NAFLD. EASL Guidelines proposed 8 and 12 kPa, respectively, as rule-out and rule-in cut-offs for advanced fibrosis. Patients with Fibroscan measurement between 8 and 12 fall in a grey zone where further investigations are recommended. We recently proposed the cACLD Risk Score to further stratify this population.

The main aim of this study was to test the diagnostic performance of NITs in a UK cohort of patients with histological diagnosis of NAFLD. Secondly, we assessed the performance of the cACLD risk score and of blood-based NITs to further stratify patients in the Fibroscan's grey zone.

Method: This is a retrospective observational study. We enrolled consecutive patients with histological diagnosis of NAFLD/NASH from January 2014 to December 2021 at the Royal Free Hospital, London, UK. We excluded patients who did not perform at least one of these NITs at time of biopsy (\pm 6 months): FIB4, NAFLD Fibrosis score (NFS), Fibroscan, APRI, cACLD Risk Score. We performed a ROC analysis to explore the diagnostic performance of NITs for cACLD (F>2). Secondly, in patients with intermediate Fibroscan results (between 8-12 kPa), we tested the diagnostic performance of cACLD Risk Score and Enhanced Liver Fibrosis test (ELF).

Results: We included 304 patients; 108 were female, median age was 55 years, and 50.6% had diabetes.

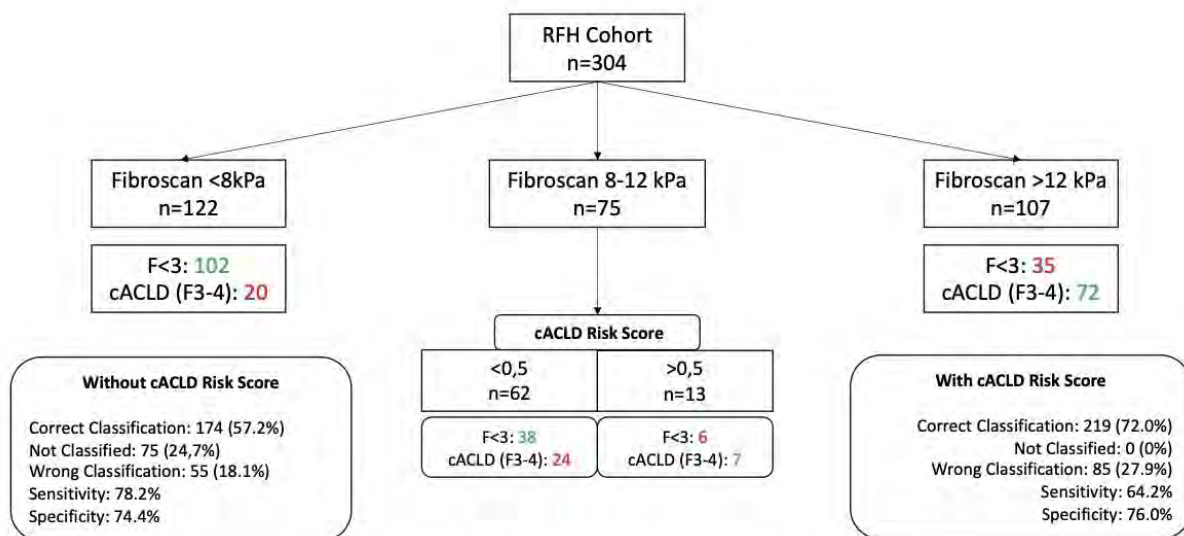
Fibroscan had the best diagnostic performance for advanced fibrosis with an AUROC of 0.77 (FIB4 0.71, NFS 0.67, APRI 0.65). EASL criteria showed a high sensitivity and high specificity for 8 kPa and 12 kPa cut-offs, respectively (Sens 83.7%; Spec 80.1%), accuracy of 57.3%, wrong classification of 18.1% and 75 patients (24.6%) in the grey zone (between 8 and 12 kPa).

The diagnostic performance of cACLD Risk Score in the Fibroscan's grey zone was suboptimal (AUROC: 0.593). The use of Fibroscan+cACLD Risk Score vs. Fibroscan alone showed a better accuracy (72% vs 57%) and a slight worsening of wrong classification % (28% vs 18%) mainly due to a higher number of false positive than false negative.

70 patients had ELF at time of biopsy. In this subgroup of patients, the use of cACLD or ELF (cut-off 11.0) in Fibroscan's grey zone showed similar results improving the classification performance of fibroscan alone (correct classification 71.4% vs 50.0%; wrong classification 28.6% vs 15.7%)

Conclusion: Our results suggest that cACLD Risk Score could be used in patients with indeterminate Fibroscan results in order to further stratify their risk of cACLD. The use of cACLD Risk Score does not impact on missed diagnosis of cACLD (false negatives) although it could lead to overdiagnosis (false positive). Patients with intermediate Fibroscan and high cACLD risk score should be still considered for further investigation (liver biopsy).

Figure:



True positive or negative, False positive or negative, Indeterminate result

P01-22 MRI in NAFLD: calculation of liver fat mass and investigation of its correlation with liver volume and steatosis grade

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Background and aims: Our MRI lab at Bioiatriki SA, Healthcare Group, Athens, Greece, participated in a study that proved the relation of PNPLA3 with steatosis. MRI data from that study, post-processed, to investigate any correlation of fat mass in liver and fat fraction, with liver volume in Non-Alcoholic Fatty Liver Disease (NAFLD) patients.

Method: A sample of 162 NAFLD patients (91 male/71 female) had liver MRI in our lab. The scanning protocol included a multi-echo (GE IQ IDEAL) and a 3D sequence. From the first sequence, fat fraction was measured in each liver segment and the liver fat fraction was considered the average from all segments. From the 3D sequence, images were reformatted in axial and coronal planes, then manual measurements of liver volumes were performed in each plane, excluding big vessels and any other non-liver structure. Total liver volume was estimated as the average measurement from the two planes. Fat mass in the liver was calculated considering fat fraction, liver volume, and triglycerides density in body temperature. Linear regression analysis was performed.

Results: In the total population, regression analysis showed a significant linear correlation of liver volume with both fat mass and fat fraction ($R^2 = 0.70/p < 0.001$ with fat mass, $R^2 = 0.45/p < 0.001$ with fat fraction). Separating data by sex, the linear correlation of liver volume for males is $R^2 = 0.59/p < 0.001$ with fat mass, $R^2 = 0.43/p < 0.001$ with fat fraction, while for females $R^2 = 0.83/p < 0.001$ with fat mass, $R^2 = 0.52/p < 0.001$ with fat fraction.

Conclusion: The relatively low linear regression of liver volume and fat fraction is a result in agreement with other previous studies. The much stronger linear regression of liver volume and fat mass is a new finding. This led us to suppose a strong correlation fat mass with steatosis grade. This analysis results (steatosis grade/average fat mass in grams: 0/58.69, 1/182.11, 2/374.01, 3/672.15), prove the assumption of strong linear correlation with $R^2 = 0.96$.

P01-23-YI The crosstalk of liver, muscle and adipose tissue transcriptomics upon exercise in patients with non-alcoholic fatty liver disease

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Background and aims: Exercise is a vital component of lifestyle management in patients with non-alcoholic fatty liver disease (NAFLD), but neither its therapeutic effect on the advanced stages, i.e. non-alcoholic steatohepatitis (NASH) including liver fibrosis amongst other, nor the underlying mechanisms have been characterized in detail. We therefore performed multi-omic tissue phenotyping of patients with NAFLD/NASH before and after a 12-week exercise regimen.

Method: Fifteen patients with histologically characterized NAFLD participated in a 12-week personalized high intensity interval training (HIIT) program combined with home-based training following international guidelines. HIIT comprised of two weekly training sessions involving warming-up (30% of intensity), repeated bouts of high intensity intervals (85%) interspersed by three minutes of active recovery (10%), and followed by cooling down (20%). At baseline and upon completion of the training, NAFLD severity was evaluated by histological readings and multiparametric magnetic resonance imaging (MRI). Differential gene sequencing analysis (DESeq2) on liver, muscle and adipose tissue RNA sequencing (RNA-Seq) were performed before and after the training, as well as the analyses of untargeted plasma metabolomics and stool metagenomics.

Results: Maximum oxygen uptake (VO₂max) increased significantly by 10.1% ($p < 0.05$) upon exercise, underscoring effect of and compliance to the exercise program. The program decreased visceral fat volume by -11.5% ($p < 0.05$) but did not affect body weight (per protocol). Exercise did not improve NAFLD as analysed by multiparametric MRI and liver histology. However, we did find marked alterations in mRNA expression in muscle and adipose tissue. More specifically, in muscle, expression of genes related to the mitochondrial respiratory chain was altered, and in adipose tissue the alterations were in genes involved in lipid transfer. In line, genes involved in adipogenesis were altered in liver, yet overall these changes were smaller than the mRNA changes in muscle and adipose tissue. Analyses of metagenomics and metabolomics are expected in Q3-2022.

Conclusion: Despite the metabolic improvement as assessed by VO₂max and gene expression in muscle and adipose tissue, exercise intervention did not ameliorate NAFLD on histology, MRI evaluation or gene expression. This raises the hypothesis that weight loss is essential for lifestyle interventions to have a meaningful effect on NAFLD, and this study warrants longer and larger controlled interventions in patients with NASH and fibrosis.

P01-25 Effect of peroxisome proliferator-activated receptor-alpha/gamma agonism on fibrosis-4 index in type 2 diabetes mellitus patients with elevated cardiovascular risk profile by sex, age, regional and ethnical differences

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Background and aims: Peroxisome proliferator-activated receptor (PPAR) agonists may be beneficial in patients with NAFLD, and dual agonists of PPAR-alpha/gamma have been suggested to reduce inflammation in patients with type 2 diabetes mellitus (T2DM). NAFLD has close bidirectional relations with T2DM, yet the effect of PPAR agonists on NAFLD has been understudied in clinical trials. This study serves as proof of concept of the PPAR agonist pathway to treat NAFLD/NASH by analyzing data from the AleCardio trial to determine if aleglitazar improves the Fibrosis-4 index (FIB-4) in T2DM.

Method: This is a post-hoc analysis of a large randomized, double-blind, placebo-controlled, multi-center trial including 7226 patients with T2DM and recent coronary syndrome (AleCardio trial). Eligible patients were randomized to receive aleglitazar or matching placebo added to standard medical care for two years. Main outcomes are changes in FIB-4 from baseline to 24 months stratified for age, gender, ethnicity and region. FIB-4 is divided into 3 categories: low = <1.30; intermediate 1.30-2.67; high ≥2.67.

Results: As expected, mean FIB-4 at baseline was lower in females (1.15 ± 0.02 SEM) compared to male patients (1.52 ± 0.01) at all timepoints $p < 0.001$. Age was a major determinant of FIB-4 scores in both genders. Aleglitazar treatment reduced FIB-4 scores in both genders within 3 months compared to placebo, although the absolute FIB-4 decrease was significantly larger in males, see figure 1. Concerning regional differences, baseline FIB-4 was significantly higher in Europe (1.57 ± 0.02) compared to all other regions studied: Asia/Pacific (1.39 ± 0.02), North America (1.33 ± 0.02) and South America (1.26 ± 0.03). For all regions FIB-4 decreased similarly by aleglitazar after 3-24 months of intervention. Concerning ethnicity, FIB-4 values at baseline were significantly different between Caucasians (1.45 ± 0.01), Asian (1.37 ± 0.2) and African-American (1.15 ± 0.06) subjects. For Caucasians and Asians the treatment effect was similar, however, treatment seemed less effective in African-Americans subjects showing a FIB-4 decrease until month 12 followed by an increases above placebo values after 24 months.

Conclusion: This post-hoc analysis in patients with T2DM and elevated cardiovascular risk profile showed that treatment with a PPAR-alpha/gamma agonist for 24 months significantly decreased FIB-4 scores compared to placebo independent from age and gender in all regions studied, although this treatment may be less effective in African-American subjects

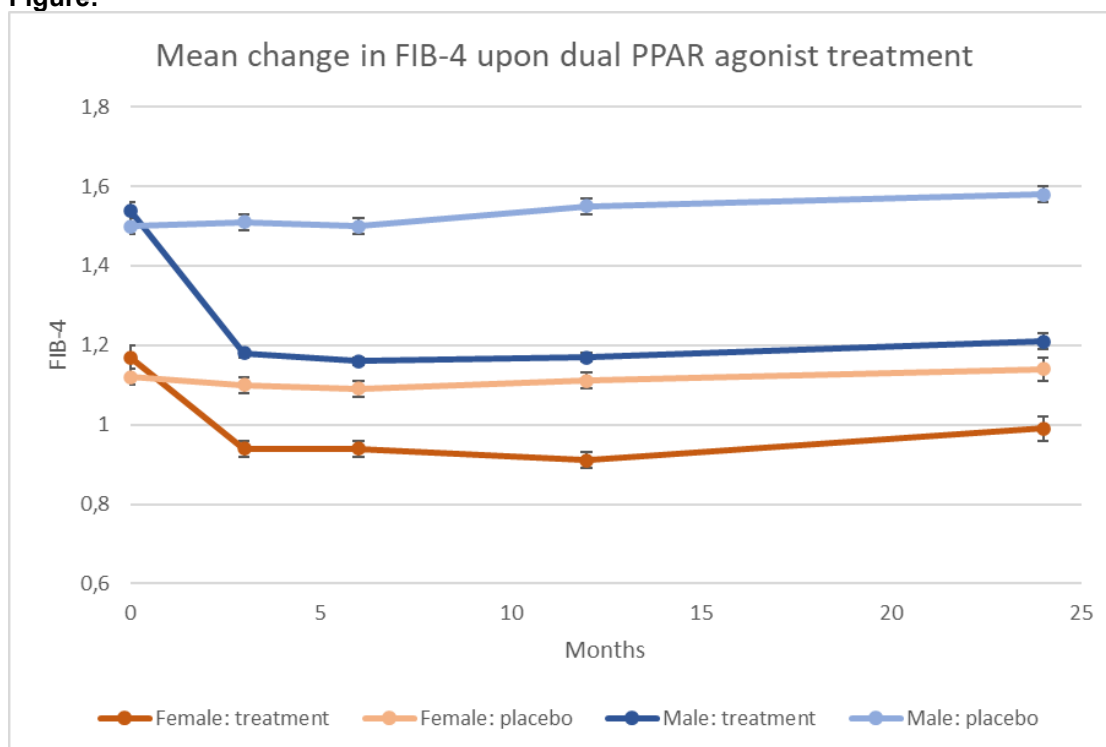
Figure:

Figure 1: mean change in FIB-4 \pm SEM from month 0 to month 24 for treatment with aleglitazar vs placebo stratified by gender.

P02-01 Non-alcoholic fatty liver disease and sarcopenic obesity

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Background and aims: Recent studies have shown that sarcopenia often accompanies NAFLD. Sarcopenic obesity in combination with progressive loss of skeletal muscle mass adversely affects a person's metabolic status, leading to a decrease in quality of life, the development of cardiovascular disease. The aim was to establish relationships between markers of inflammation, insulin resistance and sarcopenia in NAFLD patients.

Method: The study involved 192 patients with NAFLD with normal, overweight, and obese, and 96 patients without NAFLD. Conducted MRI and anthropometric survey, measured levels of AST, ALT, GGT, the degree of liver fibrosis using elastography (FibroScan), ECG. The stratification of CV risk was evaluated by SCORE scale version for countries with high risk. We determined the level of inflammatory mediators (TNF- α , IL-1, IL-6), markers (CRP, fibrinogen), endothelin -1, the thickness of the intima-media complex, presence atherosclerotic plaque and stenosis of the carotid arteries, insulin resistance index HOMA-IR for all patients.

Results: Thus, the body weight of men with NAFLD was 1.3 times higher than in the group of healthy men. The body weight of women with NAFLD was 1.5 times higher compared to the group of healthy women. There are no significant differences or trends in body length differences between healthy and NAFLD men and women with NAFLD. The BMI of men with NAFLD was 1.4 times higher than in the group of healthy men. The BMI of women with NAFLD was also 1.4 times higher than in the group of healthy women. Muscle mass of men and women with NAFLD was statistically significantly lower ($p < 0.05$) than that of sexually healthy men and women. Moreover, muscle mass in healthy men was statistically significantly higher ($p < 0.05$) than in the corresponding groups of women. Thus, healthy men had a 20.5% increase in muscle mass compared to healthy women. Higher levels of inflammation, HOMA index, and a decrease in adiponectin levels were found in patients with NAFLD and sarcopenia compared with patients with preserved muscle mass. According to the results of the study, the component composition of body weight in NAFLD changes. Compared to healthy men with NAFLD, body fat was 35.2% higher, while muscle mass and bone mass in men were 29.1% and 32.0% lower, respectively. Compared to healthy women with NAFLD, body fat was 30.2% higher, while muscle mass and bone mass in women were 17.4% and 22.7% lower, respectively. In men and women with NAFLD, strong inverse correlations ($r = 0.71$, $p < 0.001$) were found between muscle mass and hsCRP levels.

Conclusion: The pathogenesis of sarcopenia and NAFLD have common mechanisms: insulin resistance, increased level of inflammation, skeletal muscle secretion of myokines, myostatin, decreased adiponectin levels.

P02-02 Simple anthropometrics may improve non-invasive tests for the prediction of fibrosis in an elderly population: the Rotterdam study

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Background and aims: Fatty liver disease affects >30% of the global population and has rapidly become the leading cause of advanced liver disease. Advanced liver disease due to fatty liver disease is closely associated with metabolic dysfunction. Non-invasive tools to assess liver health in the general population has either limited availability or poor diagnostic performance, especially in the elderly. Therefore, we aimed to investigate whether simple anthropometrics could improve currently available risk stratification strategies for the detection of fibrosis.

Method: We used data from the Rotterdam Study, a large, European population-based cohort in persons 55 years and older. We assessed hepatic steatosis and fibrosis (defined as a liver stiffness ≥ 8.0 kPa) by ultrasound and transient elastography. Participants without viral hepatitis, alcoholic liver disease or history of heart failure were included. First, we assessed the diagnostic accuracy of BMI and waist circumference to predict fibrosis as compared to the FIB-4. Next, we modified the FIB-4 by adding waist circumference and/or BMI. Subgroup analysis included stratification for sex, age ≥ 65 and metabolic dysfunction (BMI ≥ 25 , diabetes or ≥ 2 minor criteria).

Results: Among the 4,080 included participants (aged 67 ± 8 , 44% male), 33.2% had steatosis and 5.6% had fibrosis. FIB-4 (AUC 0.62) did not have better discrimination for fibrosis compared to simple anthropometrics such as BMI (AUC 0.61) and waist circumference (AUC 0.66). In subgroup analysis, we consistently demonstrated similar performance of FIB-4 compared to simple and readily available anthropometrics. However, modifying the FIB-4 significantly increased its discriminating value. For example, adding waist circumference and BMI to the FIB-4 improved its performance (AUC 0.62 vs 0.71, $p < 0.001$). Moreover, using the individual components of the FIB-4 together with BMI and waist circumference, the AUC significantly increased to 0.74, whereas optimization of the FIB-4 without BMI and waist circumference yielded a significantly lower AUC of 0.69 ($p < 0.001$).

Conclusion: Among the elderly general population, FIB-4 poorly discriminates between individuals with and without liver fibrosis. Adding anthropometrics to FIB-4 improved its discriminative value and adequate redesigning of the FIB-4 may yield better results than the FIB-4 or anthropometrics alone.

P02-03 Prediction of fibrosis progression and clinical outcomes with non-invasive tests in 10 years follow-up of patients with non-alcoholic steatohepatitis

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Background and aims: Fibrosis stage is the most important prognostic factor in non-alcoholic fatty liver disease (NAFLD). The gold standard tool for staging fibrosis in NAFLD is liver biopsy, which is a difficult tool to use on follow-up evaluations. Non-invasive tests (NITs) were developed to first stratify patients at risk for advanced fibrosis but not validated for follow-up or treatment response assessments. The aim of this study was to evaluate liver fibrosis progression, NITs variations over time and their correlations with clinical outcomes (hepatic decompensation; hepatic and extra-hepatic neoplasm; cardiovascular events and mortality).

Method: Retrospective cohort of 138 patients with biopsy proven non-alcoholic steatohepatitis (NASH), followed in a tertiary hospital. Patients underwent regular clinical and physical assessment; laboratory examinations and NIT assessments (FIB-4 and transient elastography). Fibrosis progression was estimated using transient elastography. NIT variations over time were compared with the development of clinical outcomes.

Results: 138 patients were analyzed. Median age was 65 years and median body mass index was 32Kg/m² at diagnosis. 77 patients (55%) had diabetes and 82 (59%) had hypertension at diagnosis. 56 patients (40%) had advanced fibrosis (\geq F3) and 18 (13%) of them had cirrhosis at biopsy. Median time of follow-up was 10 years. 119 patients performed fibroscan at the end of the follow-up. 59 patients progressed to cirrhosis (49, 6%). Initial NAFLD activity score (NAS) was statistically associated with fibrosis progression. 24 patients (17%) developed a clinical outcome. Fibrosis stage at diagnosis was associated with cirrhosis decompensation but not associated with cardiovascular events. Fibrosis progression assessed with elastography (>11 , 5kPa) was associated with portal hypertension development. FIB-4 elevation during follow-up (>2 , 7) was associated with cirrhosis decompensation.

Conclusion: High-risk NAFLD patients have a high prevalence of fibrosis progression and clinical outcomes. NITs such as FIB-4 and transient elastography might be useful tools for evaluation of disease progression and risk of hepatic decompensation. More prospective studies are needed to better define NITs cut-offs for risk of clinical outcomes.

P02-04 The HDL proteome serves as a mirror for liver dysmetabolism and can delineate healthy from unhealthy obesity

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Background and aims: High-density lipoprotein (HDL) particles, and their associated proteins (>100), are predominantly liver-derived. We have shown that the HDL proteome is enriched with hepatic-derived pro-inflammatory proteins, and mirrors changes in the liver proteome, after prolonged high-fat feeding in mice^{1,2}. In this study we hypothesized that HDL protein and function is compromised in human obesity contributing to deteriorating cardiometabolic health.

Method: Patients with obesity attending St. Vincent's University Hospital, were categorized as metabolically healthy obesity (MHO, $n = 45$) or metabolically unhealthy obesity (MUO, $n = 65$) based on NCEP-ATPIII guidelines. Normal weight (NW, $n = 129$) individuals served as controls. Efflux function of small (ABCA1-dependent) and large (ABCA1-independent) HDL particles was determined by measuring ³H-cholesterol efflux from J774 macrophages treated \pm cAMP. Paraoxonase-1 activity was determined enzymatically. HDL was isolated from serum by fast protein liquid chromatography (FPLC) and protein content determined by mass spectrometry ($n = 10$ NW, $n = 7$ MHO and $n = 12$ MUO).

Results: ABCA1-independent HDL efflux capacity and serum PON1 activity were significantly reduced in both MHO and MUO groups relative to NW controls independent of metabolic health status. The HDL proteome was profoundly and progressively modulated in obesity with increased association of complement proteins C2 and C6, C-reactive protein, heparin co-factor 2 and serum amyloid-P and reduced association of apolipoproteins (ApoA1, ApoC-III, ApoA-IV, ApoD), immunoglobulins and PON1 on MUO-HDL relative to NW-HDL. A metabolic HDL index (MHI) score was generated from the HDL proteomic data and significantly and incrementally decreased in MHO and MUO groups relative to NW group. Furthermore, MHI negatively correlated with body mass index (BMI) ($r = -0.738$, $p < 0.001$), diastolic blood-pressure ($r = -0.455$, $p < 0.05$), glucose ($r = -0.549$, $p < 0.01$), and positively correlated with HDL-C ($r = 0.486$, $p < 0.01$) and ABCA1-independent efflux ($r = 0.389$, $p < 0.05$).

Conclusion: Adverse changes in HDL protein composition and function in human obesity likely contributes to exacerbated risk of cardiovascular complications. HDL proteomic composition is a powerful biomarker of metabolic health status and may serve as a critical biomarker of liver dysmetabolism but within an easily accessible biofluid.

References

- 1 O'Reilly, M. *et al. Circulation* (2016).
- 2 Kajani, S. *et al. Mol Metab*, 101425 (2021).

P02-05-YI Characterization of cognitive function in patients with non-alcoholic fatty liver disease and association with the risk of progressive non-alcoholic steatohepatitis

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) includes a wide spectrum of liver damage ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), a condition at high-risk of liver cirrhosis progression. Cognitive impairment has been associated to different cardiometabolic diseases, including NAFLD. We sought to determine the cognitive profile of patients with NAFLD and to investigate the potential association of impaired cognitive function with non-invasive score of “at risk NASH”.

Method: A total of 159 patients with a diagnosis NAFLD by ultrasound (median age 53, 44-63 years; 69 males and 85 females; median body mass index 29.3, 26.2-32.4 kg/m²; type 2-diabetes mellitus [T2DM]: n = 38, 24.5%) underwent neurocognitive testing with the Repeatable Battery for Assessment of Neuropsychological Status (RBANS); the questionnaire evaluates the overall cognitive function and five subdomains assessing the immediate memory, visuospatial and constructional function, language function, attention, and delayed memory. Score values <90 were indicative of cognitive impairment. All patients underwent liver stiffness + controlled attenuation parameter (CAP) assessment. The risk of progressive NASH was assessed by FibroScan-AST (FAST) score.

Results: An impaired overall cognitive function was observed in 81 (50.9%) patients (median score value: 89, 77-101), with the most prevalent deficit in immediate memory (n = 101; 63.5%), followed by delayed memory (n = 85, 53.5%), visuospatial and constructional function (n = 67; 42.1%), language function (n = 66; 41.5%), and attention (n = 26; 16.4%). Patients with impaired overall cognitive function showed higher FAST score values as compared to patients with average cognitive status (0.25, range 0.14-0.41 vs. 0.19, range 0.08-0.36; p = 0.044). The degree of attention impairment resulted significantly associated to the presence of T2DM (OR = 2.13, 95% CI 1.28-3.52; p = 0.003) and obesity (OR = 1.94, 95% CI 1.13-3.32; p = 0.015). In obese NAFLD patients a FAST score value >0.35 resulted significantly associated to impaired cognitive function (OR = 3.59, 95% CI 1.04-12.45, p = 0.044). FAST values progressively increased according to the degree of impairment in language (p = 0.001) and attention (p = 0.027).

Conclusion: We observed an impaired cognitive function in a consistent proportion of patients with NAFLD, particularly in those with obesity and T2DM; the severity of cognitive impairment progressively increases in patients with “at risk NASH”.

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P02-07 Impact of dietary intake on the risk for significant fibrosis in patients with MAFLD

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Background and aims: Metabolic dysfunction-associated fatty liver disease (MAFLD) includes a heterogeneous spectrum of metabolic abnormalities associated with the presence of hepatic steatosis, which are involved in liver disease progression. To date, healthy lifestyle modifications based on diet and physical activity are a cornerstone for the management of these patients. The aim of this study was to investigate the impact of dietary components on the severity of liver damage in patients with MAFLD.

Method: A total of 149 patients with MAFLD were enrolled between 2016 and 2021. Anthropometric, clinical and biochemical parameters were collected at the time of enrolment. Liver fibrosis was assessed by transient elastography (Fibroscan®530) and classified in 5 groups (from F0 to F4) according to established cut-off. Eating habits were evaluated through the European Prospective Investigation into Cancer and Nutrition Food Frequency Questionnaire and Low Carbohydrate, high-protein (LCHP) score was computed.

Results: Overall, median age was 56 years (IQR 46;63) and 61.07% of patients were male. In addition, 32 subjects had advanced fibrosis (F2-F4). Patients with F2-F4 showed higher levels of transaminases, glucose, total cholesterol and triglycerides compared to those with F0-F1. Concerning dietary intake, patients with F2-F4 in comparison to F0-F1 group were characterized by a lower energy intake (1418 kcal vs. 1709 kcal, $p = 0.008$). Specifically, these patients showed a significant higher protein intake and LCHP score (20.2% vs. 18.6%, $p = 0.050$ and 15 vs. 13, $p = 0.012$, respectively), while less fat intake (34.3% vs. 36.6%, $p = 0.008$) was observed. Interestingly, a multivariate logistic regression analysis revealed that the highest tertile of protein intake and the LCHP score resulted significantly associated to the risk for F2-F4 liver fibrosis (OR: 3.24 (1.00;10.43), $p = 0.049$ and OR: 1.23 (1.06;1.44), $p = 0.006$, respectively) adjusted for sex, age, body mass index and diabetes.

Conclusion: In conclusion, higher protein intake and LCHP score were associated with fibrosis severity in patients with MAFLD. However, further research is needed to better understand the pathophysiological mechanism of protein intake, as well as the role of other environmental variables that may modulate each individual's response.

Italian Ministry for Education, University and Research (MIUR) under the programme "Dipartimenti di Eccellenza 2018-2022" Project code D15D18000410001.

P02-10 Interactive role of non-invasive assessment of insulin resistance and liver fibrosis in the prediction of cardiovascular events in patients with MAFLD

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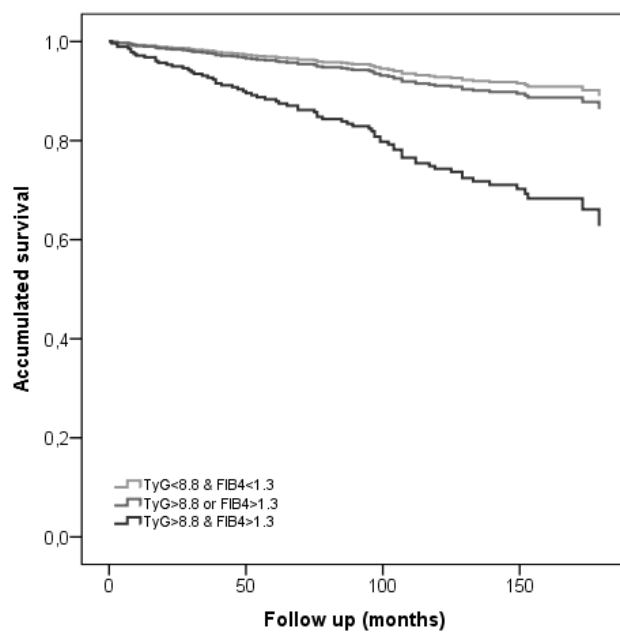
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Background and aims: Cardiovascular events (CVE) are the most common cause of morbidity in patients with MAFLD. Liver fibrosis (LF) is a demonstrated independent risk factor of CVE in patients. However, the interplay of the non-invasive assessment of LF and classic scale cardiovascular risk (CVR) and insulin resistance (IR) remains obscure in the clinical practice. Our aim is to evaluate the interactive role of LF, classic scale CVR and IR evaluation using non-invasive methods in the prediction of CVE in patients with MAFLD.

Method: Retrospective longitudinal cohort analysis of patients evaluated in a single teaching hospital between 1997 and 2002 (CUN-Vascular). The exclusion criteria were an age <50 or >70 years old, an HSI score <36 and the diagnosis of any CVE, diabetes mellitus or chronic liver disease other than MAFLD prior to recruitment. The incidence of myocardial ischemia or cerebrovascular stroke was the study outcome. Age, sex, alcohol consumption (yes/no) and variables to calculate CVR, IR and LF were recorded. CVR was assessed using the SCORE scale, while Fibrosis index-4 (FIB4) and Triglyceride to Glucose (TyG) ratio were used for the evaluation of LF and IR. Cut-off values of FIB4>1.3 and TyG>8.8 were used in the evaluation of patients, as previously described. SPSS 20.0 was used in the statistical analysis.

Results: A total of 951 patients were analyzed. Mean age was 59 ±5 years, with a 37 % of women. A 40 % of patients referred alcohol consumption. The mean SCORE was 4 ± 7 %, with 13 %, 49 % and 37 % in respective risk subgroups. Mean FIB4 was 0.95±0.46 points with 115 individuals over 1.3 points. Mean TyG was 8.5 ± 0.5 with 255 patients over 8.8 points. The mean follow-up was 102 ± 58 months. Incident CVE were reported in 69 patients (Coronary n = 48 vs. Neurological n = 21). Patients at high CVR, TyG and TyG >8.8 and alcohol consumption were statistically significant in the univariate prediction of CVE. FIB4 and FIB4>1.3 did not show prediction capacity on their own. Nevertheless, the interaction between FIB4>1.3 and TyG>8.8 was statistically significant after adjustment by TyG (HR 2.67 CI 95 % 1.02-6.95) and after adjustment by high cardiovascular risk, alcohol consumption and TyG (HR = 2.80 CI 95 % 1.07-7.35). Finally, the subgroup of patients with TyG >8.8 and FIB4 >1.3 had a statistically significant higher risk of CVE during follow-up in an adjusted model (HR = 3.95 CI 95 % 1.52-10.19).

Conclusion: The combined non-invasive assessment of insulin resistance and liver fibrosis with TyG index and FIB4 might point out a very high cardiovascular risk subgroup of patients among those with non-invasively suspected MAFLD. Prospective analysis should be performed to validate these results

Figure:

P02-12 Impact of renaming NAFLD to MAFLD in a single italian center

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Background and aims: Metabolic Associated Fatty Liver Disease (MAFLD) is the novel definition and the new paradigm of NAFLD introduced in 2020, but its clinical and health public implications remain under evaluation. The aims of this study are to analyze the prevalence of MAFLD and NAFLD and compare them in a well-defined cohort of patients with liver steatosis.

Method: A cross-sectional study was conducted in a single Liver Unit in Italy. Clinical, laboratory and imaging data were collected. NAFLD and MAFLD were defined according to international expert consensus.

Results: A total of 527 patients with ultrasound steatosis were included. The mean age was 64 ± 18 years (58.8% male). The mean body mass index (BMI) was 29 ± 5 kg/m². The percentage of participants with arterial hypertension, type 2 diabetes mellitus and dyslipidemia were 59.28%, 22.82% and 46.76%, respectively. The median liver stiffness and CAP (Controlled Attenuation Parameter) values were, respectively, 6.5 ± 4.6 kPa and 287 ± 72 dB/m. MAFLD was present in 309 (69.1%) patients, while NAFLD in 138 (30.9%) of them. Compared with NAFLD subjects, MAFLD patients are older ($p = 0.0001$), with higher frequency of arterial hypertension ($p = 0.000$), had higher FIB-4 ($p = 0.0001$), APRI ($p = 0.023$) and NAFLD fibrosis score ($p = 0.05$) values. No significant differences in liver stiffness, CAP values and transaminases were observed.

Conclusion: Metabolic-associated fatty liver disease is a highly prevalent condition in this well-defined cohort. MAFLD seems to be superior to NAFLD definition for identifying fibrosis at-risk patients.

P02-13 Prevalence and risk factors for non-alcoholic fatty liver disease in patients with polycystic ovary syndrome: a systematic review, meta-analysis, and meta-regression

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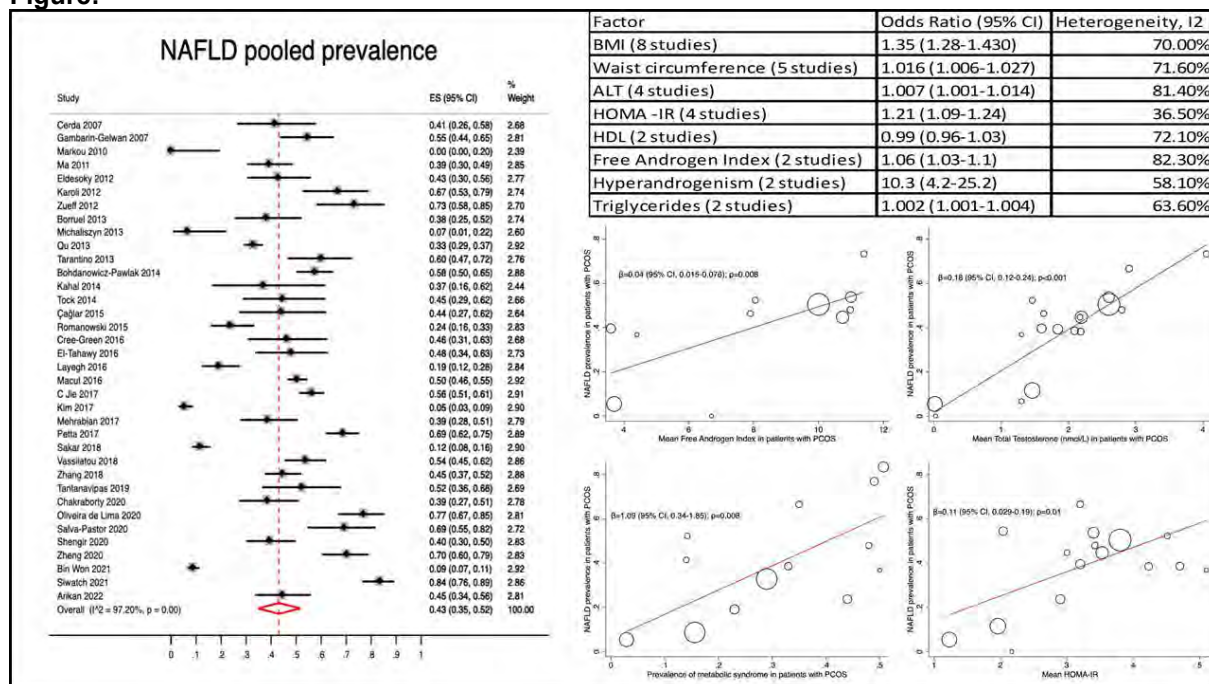
Background and aims: Polycystic ovary syndrome (PCOS) has detrimental effects on different metabolic tissues, increasing adiposity and insulin resistance. These effects may increase the risk of non-alcoholic fatty liver disease (NAFLD). However, the burden of NAFLD in PCOS has been overlooked. Therefore, we performed a systematic review (SR), meta-analysis (MA) and metaregression (metareg) to assess NAFLD's prevalence and risk factors in patients with PCOS.

Method: A literature search was performed in MEDLINE, Scopus, and Scielo. For this SR, the articles considered eligible for inclusion were those examining either the occurrence or risk factors of NAFLD among patients with PCOS. Two types of meta-analyses were performed. First, we performed a MA of proportions to estimate the prevalence of NAFLD among PCOS patients. Second, we performed MA of precalculated adjusted odds ratios extracted from the included studies to examine the NAFLD risk factors in PCOS. Finally, we performed a random-effects metareg to estimate the coefficients, β , which indicate how the estimated prevalence changed with changes in prespecified variables.

Results: We identified 815 articles from the database searches. Of these, 36 were included. All articles were included in the proportions MA. 12 studies were included in the risk factors MA. Using a MA of proportions with a random-effects model, we found a pooled NAFLD prevalence of 43% (95% CI, 35-52) with high heterogeneity ($I^2 = 97.2\%$) (Fig). Risk factors MA found that BMI, waist circumference, ALT values, HOMA-IR values, free androgen index levels, hyperandrogenism, and triglycerides were associated with significantly higher risk-adjusted odds of NAFLD among patients with PCOS (Fig). Metareg showed that rises in NAFLD prevalence were mediated through increases in metabolic syndrome prevalence and higher levels of HOMA-IR, free androgen index, and total testosterone (Fig).

Conclusion: This is, to our knowledge, the first comprehensive MA examining the burden and risk factors for NAFLD among PCOS patients. The results presented herein portray a worrying scenario with a high prevalence of NAFLD (43%) among PCOS patients with several metabolic and PCOS-specific factors influencing its occurrence. As the burden of NAFLD appears to be high, screening programs may aid in detecting metabolic-associated fatty liver disease and prevent its consequences in a population where this condition has been commonly overlooked.

Figure:



P02-14 Non-alcoholic fatty liver disease related knowledge among Egyptians: an exploratory cross-sectional study

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Background and aims: non-alcoholic fatty liver disease (NAFLD) has become the major cause of chronic liver disease because of the global obesity epidemic. Available data suggest that Egypt has one of the highest prevalence rates, compared to a global prevalence of roughly 25%. Several studies have found a paucity of NAFLD awareness and knowledge among secondary care practitioners. The detection of risk factors, presenting symptoms, investigations, and diagnosis of NAFLD as a spectrum of illness, ranging from simple NAFLD to more significant NASH and cirrhosis, was typically poor. The aim of this study is to ascertain general awareness of NAFLD and its risk factors in the general population.

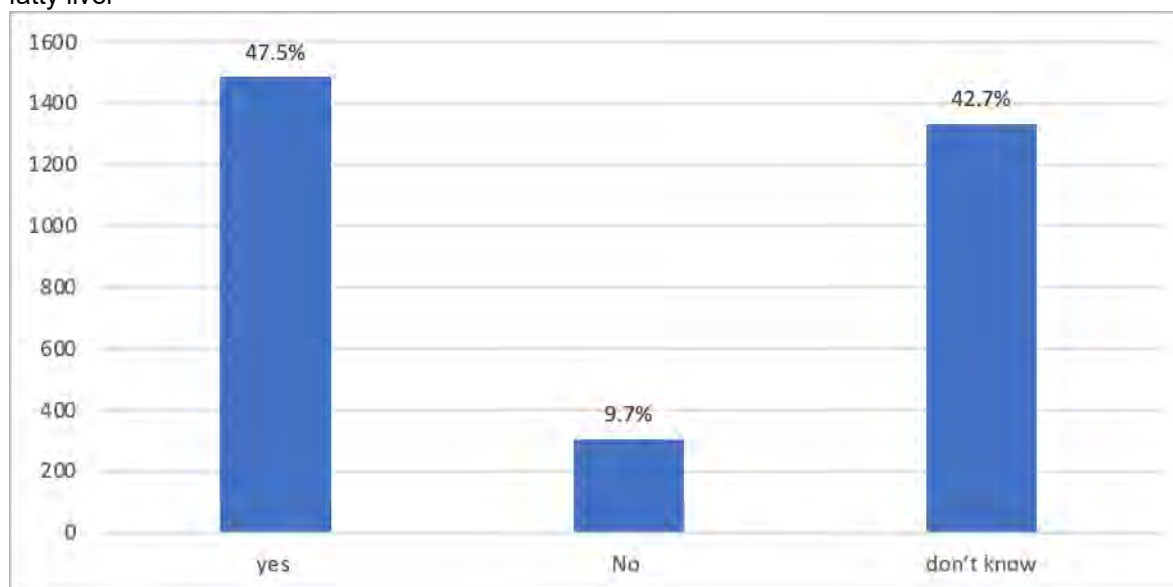
Method: The current study was performed to assess Egyptian's fatty liver related knowledge and attitudes after receiving ethical approval from Medicine Cairo University. Inclusion criteria were 18 years or older, who were willing to participate in the study. Healthcare workers and NAFLD patients were excluded. The total collected questionnaires were 1124 printed copy and 2000 online forms included four sections: Socio-demographic characteristics, fatty liver related knowledge: composed of a total of 31 items that addressed the definition, the symptoms, and complications (10 questions), risk factors (14 questions), and prevention and treatment (7 questions). The total raw score (if all answers were correct) was 31.

Results: The current study included 3124 participants. 57% of them were females, while one quarter of them belonged to the age group of 18-29 years, about one third of participants were diabetic (32.5%). Knowing that fatty liver is due to the deposition of fat, given by 34% of participants while only 10.8% assuming that patients with fatty liver are asymptomatic. More than half of the studied subjects believed that they were at risk of acquiring fatty liver (59%), as displayed in Figure 1. Fatty liver related to hypercholesterolemia and increased fat intake were the highest correct responses at 60% and 54% as predisposing factors. More than 97% of participants did not believe there was an effective treatment for fatty liver, even though nearly 90% of them believed it was a preventable disease. Weight reduction was acknowledged by 81% of them as an effective preventive measure, and out of score 31 of all correct answers; 10 was the media with 8 as interquartile ratio (IQR)

Conclusion: The current study found that there was a lack of an informed response towards NAFLD knowledge, risk factors, preventive measures, and treatment among Egyptians despite of growing prevalence of NAFLD, Health care systems should focus on NAFLD to increase awareness.

Figure:

Figure (1) % distribution of the enrolled participants by their attitude towards being at risk of acquiring fatty liver



P02-15 Impact of sexual dimorphism and aging on NAFLD severity and advanced fibrosis

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Background and aims: The most important risk factors for Non-alcoholic fatty liver disease "NAFLD: are male gender, age, obesity, insulin resistance and the cardiometabolic alterations that define the metabolic syndrome. Our aim was to study the Impact of sexual dimorphism on degree of severity and progression of NAFLD.

Method: We studied 363 NAFLD patients aged, both sexes, with BMI ≥ 18.5 kg/m² initially diagnosed with a high probability of NAFLD using ultrasound, clinical data was collected including patient demographics, anthropometric measurements. Lipid profile, liver enzymes: alanine aminotransferase, aspartate aminotransferase, gamma- glutamyl transpeptidase, serum albumin, and bilirubin were measured. Fasting blood glucose and insulin (HOMA-IR was calculated), HbA1c%. Assessment of liver steatosis and fibrosis by fibro scan.

Results: A total of 363 NAFLD patients were included in the present study; 53.2% were men, BMI was 33.6 ± 8.7 kg/m², 50.8% of 45 years or older participants were women, while 57.1% are men in a group aged less than 45 years. Significantly BMI, and WC were higher in older people, and so fasting blood sugar, insulin, HbA1c%, and hand on hand HOMA-IR. Regarding total lipid profile; it was remarkably matched in patients younger or older in age, Older age group recorded higher liver fat content (cap) and fibrosis assessment (kPa) was significantly more advanced ($p < 0.001$).

Matched age 170 NAFLD Women compared to 193 men, showed a significantly higher BMI, but lesser WC ($p = < 0.001$), almost matched in their fasting glucose, insulin, and HbA1c%, TGs were significantly higher in men and so lower HDL-c. Liver fat content were significantly higher in men ($p = 0.017$), while liver fibrosis were in general almost matched (6.4 ± 2.5 in females and 6.8 ± 2.8 in males), but 5.8% of male patients had advanced fibrosis (F4), while only 4.5% had so with p value of < 0.001

There was a statistically significant difference between female and male below under 45 years regarding the fibrosis and steatosis, while they were matched when both aged 45 years or above. Regarding diabetes as a risk factor, number of prediabetics and diabetics were matched in both groups and subgroups, males had higher triglycerides level in younger age, while females in all age groups had significantly higher HDL-c.

Surprisingly when we compare below and above 45 years patients in both genders, we found that; older females had highly significant steatosis and advanced fibrosis ($p = < 0.001$), while older males had matches steatosis to younger ones ($p = 0.894$) and significant advanced fibrosis.

Conclusion: Ageing is a main risk factors for advanced fibrosis, females are protected from disease progression even at the presence of obesity and insulin resistance by her estrogen, and with subsidence of her protected hormones, she developed rapid progressive liver fibrosis and catching the severity of the male NAFLD.

P02-16 Metabolic associated fatty liver disease: association between the three different criteria definition and hepatic and cardiovascular disease

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Background and aims: Metabolic associated fatty liver disease (MAFLD) is defined by hepatic steatosis and one criteria among: (1) body mass index (BMI)>25 kg/m²; (2) type 2 diabetes (DM); (3) metabolic dysregulation in lean subjects (BMI<25). MAFLD exposes to hepatic and cardiovascular (CV) disease. Aim: to evaluate the different impact of the three features of MAFLD on the hepatic and CV disease.

Method: 688 subjects (69% males, mean age 53±12 ys) were classified as MAFLD and enrolled in two Italian liver units. Liver disease was evaluated by ultrasound (US) to detect and grade hepatic steatosis and by Fibroscan to diagnose advanced fibrosis (≥F3) (liver stiffness measurement, LSM>8.7/7.2 kPa M/XL probe). CV disease was evaluated by carotid Doppler US and radiofrequency (carotid plaques; carotid stiffness as pulse wave velocity (PWV)), echocardiography (increased epicardial adipose tissue: EAT>9.5/7.5 mm M/F).

Results: Eighty% of patients had BMI>25 without other metabolic alterations (group 1), 2% had DM without other metabolic alterations (group 2), 13% had BMI>25+DM (group 3) and 5% had BMI<25 with metabolic dysregulation (group 4). Because of the small number of pure DM, we considered group 2 and 3 together (2a). By comparing group 1 and 4, obese and lean patients had the same severity of liver (severe steatosis 16% vs 12%, p = 0.63; advanced fibrosis 8% vs 3%, p = 0.72) and CV disease (plaques 30% vs 44%, p = 0.129; increases EAT 27% vs 33%, p = 0.53; PWV 7.8±1.9 vs 7.9±1.9 m/s, p = 0.77). When comparing patients with BMI>25+DM with simple obese or lean, an increased prevalence of severe steatosis (30%, p = 0.006 and p = 0.06) and ≥F3 (31%, p < 0.001 and p < 0.001) was evident in group 2a vs the other two, as well as higher prevalence of increased EAT (40%, p = 0.02) and PWV values (8.7±2 m/s, p < 0.001) in group 2a compared only to 1 but not 4. In multivariate analysis (adjusted for age, sex, smoking and statins use), BMI>25+DM remained an independent risk factor for severe steatosis (OR 2.4, CI 95 1.5-4.1), ≥F3 (OR 3.6, CI 95 1.9-6.6) and carotid plaques (OR 1.8, CI 95 1.1-3.0).

Conclusion: Among all features of MAFLD, the coexistence of obesity+ DM seems to play the major role in the onset of hepatic and CV disease. Notably, lean subjects with metabolic dysregulation present the same hepatic of obese subjects and the same CV alterations of obese+DM ones. This stresses on the need of a careful screening for complications and metabolic alterations in MAFLD patients, even if lean.

P02-17 Type 2 diabetes and religious fasting: effects on metabolism and liver steatosis

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Background and aims: Religious fasting during the holy month of Ramadan requires abstaining from food and liquids during the day. After sunset, it is allowed to consume food and drinks in defined periods. Fasting results in a reduced and timed calorie intake and can be regarded as a form of intermittent fasting. Type 2 diabetes (TD2) is a significant risk factor for the development of non-alcoholic steatohepatitis (NASH), the progressive form of non-alcoholic fatty liver disease (NAFLD). Recently several studies suggested some benefits of interval fasting for patients with TD2 and NASH. Here, we aimed to characterize the impact of a one-month interval fasting period on liver health, glucose and lipid metabolism in a cohort of T2D patients.

Method: 20 muslim patients from a German metropolitan region were included. The status of the liver was assessed by Fibroscan®, including the measurement of the controlled attenuation parameter (CAP). Additionally to anthropometric measurements, patients' blood samples were collected at the beginning and end of the four-week fasting period to quantify key parameters of liver injury, lipid and glucose metabolism.

Results: Following the fasting period transient elastography measurements (8.0 ± 1.2 vs. 8.6 ± 1.6 before/after fasting) and CAP values (311.4 ± 9.6 vs. 302.6 ± 10.4 before/after fasting) showed modest changes. In contrast, LFTs, apoptosis marker (M30), and adiponectin significantly decreased after the fasting period. Serum levels of triglycerides were lower following the fasting period. Glucose levels did not change significantly, but serum levels of C-peptide and insulin increased following the fasting period. In addition, the patients experienced a significant weight loss at the end of the fasting month.

Conclusion: In this cohort of T2D patients, we demonstrated that a 4-week intermittent fasting period resulted in an improvement in different serum parameters associated with glucose, liver, and lipid metabolism.

P02-19 Missed opportunities to co-diagnose alcohol-related liver disease in patients diagnosed with non-alcoholic fatty liver disease

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Background and aims: Excess alcohol consumption is a major cause of liver morbidity and mortality worldwide. The other major cause of liver disease is non-alcoholic fatty liver disease (NAFLD); in order to qualify for this diagnosis, individuals must report less than 20g and 30g of alcohol consumption per day in women and men, respectively. In Ireland, 69% of obese or overweight individuals report binge drinking, highlighting the need to assess for alcohol excess, be it through alcohol history or formal screening tools. The AUDIT-C screening tool is validated to identify hazardous patterns of alcohol consumption associated with harm and a score ≥ 5 identifies individuals at risk of alcohol harms. AUDIT-C scores are taken alongside all patients undergoing transient elastography for liver disease staging at our unit. The purpose of this study was to determine the agreement between alcohol history from our outpatient clinic and AUDIT-C score administered by specialist liver nurses in patients with a diagnosis of NAFLD.

Method: Hospital records including clinic letters from patients who had undergone AUDIT-C screening were retrospectively reviewed to determine whether alcohol use was correctly assessed at the time of clinic review.

Results: There were 265 patients with complete AUDIT-C scores; 29.2% were female; 43.8% ALD, and 24.7% NAFLD. Only 31.7% (84/265) of patients had clinic letters with documented alcohol consumption. Of these, 57.1% (48/84) of clinic letters accurately identified alcohol risk, 7.1% (6/84) overestimated, and 11.9% (10/84) underestimated alcohol risk when compared with nurse administered AUDIT-C screening. There was good inter-rater agreement between clinic letters and nurse administered AUDIT-C screening (Cohen's Kappa 0.62 [95% CI; 0.45, 0.79]). 34.0% (36/106) of known NAFLD cases could have had a revised diagnosis of concomitant ALD if an alcohol screen had been done in clinic.

Conclusion: There was poor documentation of alcohol screening in clinic letters, however when performed, there was reasonable identification of alcohol risk. The opportunity to co-diagnose ALD was missed in almost a third of NAFLD patients, highlighting the need for mandatory alcohol screening in this group.

P02-20-YI NAFLD and liver stiffness predict clinical events and chronic kidney disease in patients with type-2 diabetes

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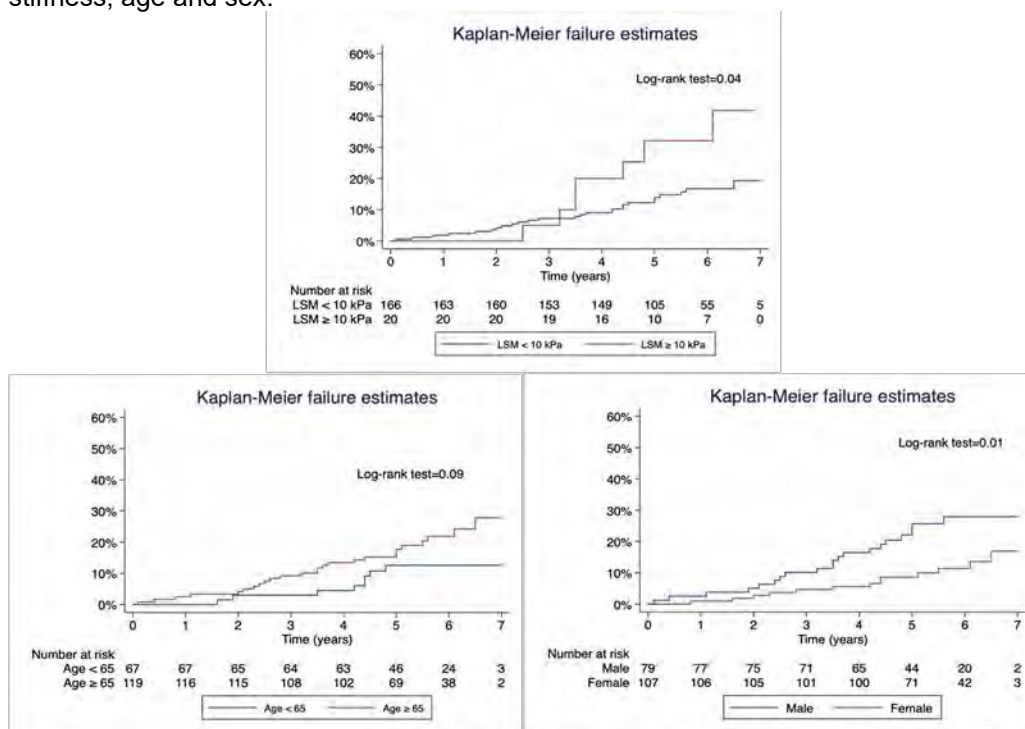
Background and aims: Non-alcoholic fatty liver disease (NAFLD) is associated with poorer glycemic control and a higher risk of type-2 diabetes (T2D) complications, extrahepatic and cardiovascular disease (CVD). Our study aim was to evaluate the association between NAFLD, T2D complications, and the development of overall clinical events (OCE) (CV, liver-related, and mortality) in patients with T2D.

Method: Prospective single-centre study comprising T2D subjects with no history of CVD and non-T2D matched controls. Patients were selected from the Outpatient Diabetes Clinic of Vall d'Hebron Hospital and related primary care centres.

Results: 186 diabetics and 57 controls were included. Amongst T2D, 124/186 subjects had NAFLD (66.6%). T2D-NAFLD subjects showed a heavier metabolic burden and a higher median liver stiffness (5.6 kPa [4.5-7.3] vs 4.8 [4.2-5.8]; $p = 0.004$) compared to non-NAFLD diabetics. During a median follow-up of 5.6 years, 33 (17.7%) T2D patients developed OCE vs 4 (7.0%) controls ($p = 0.049$). No differences were found for OCE between NAFLD and non-NAFLD diabetics (16.9% vs 19.4%; $p = 0.68$). CV was the most reported outcome and only one liver event occurred. NAFLD diabetics showed more often chronic kidney disease (CKD), whereas T2D complications and subclinical CVD rate were similar. A higher liver stiffness, older age and male gender were independently associated with OCE amongst the entire T2D population and NAFLD diabetics (Figure).

Conclusion: NAFLD and liver stiffness were associated with CKD and clinical outcomes in diabetics, respectively. A hepatic evaluation is recommended to identify high-risk T2D patients that would benefit from early referral to specialized care.

Figure: Kaplan-Meier survival curves for overall clinical events (OCE) in T2D patients according to liver stiffness, age and sex.



P02-22 Prevalence and risk factors of NAFLD fibrosis amongst penitentiary population in Catalonia. Preliminary results from the PRISONAFLD study.

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Background and aims: The prison population has experienced a progressive increase in the prevalence of chronic non-communicable diseases, especially metabolic syndrome (MetS). Non-alcoholic fatty liver disease (NAFLD) is considered the hepatic manifestation of MetS and therefore, a significant proportion of imprisoned persons are expected to suffer from it, yet there are no available prevalence data of NAFLD in this population. We aimed to describe the prevalence of NAFLD and related significant fibrosis amongst the penitentiary population in Catalonia.

Method: Cross-sectional observational study involving 8 penitentiary centres in Catalonia with a target population of 7,000 inmates. Participants had at least one metabolic criterion defined by NCEP-ATP III and were at closed regimen. Patients with other concomitant liver diseases and those with risk alcohol consumption assessed by AUDIT test were excluded. High likelihood of significant liver fibrosis was defined as a liver stiffness measurement (LSM) ≥ 8 kPa by transient elastography (TE). Enhanced liver fibrosis (ELF) score and PNPLA3 and TM6SF2 genotypes were also investigated. Multivariate regression analysis was performed to identify predictors of significant fibrosis.

Results: From the 3,263 subjects screened for metabolic risk factors between September 2021 and March 2022, 810 (24.8%) have been identified. Data of the remaining 360 subjects studied with ET after exclusions and losses are presented. Mean age was 46.0 years (SD 12.4) and 80.2% were men. Ethnic background was diverse, with 60.3% caucasians, 21.1% hispanics, 15.6% africans and 3.0% asians. Inmates from outside Spain were 50.6%. Median BMI was 29.0 kg/m² (IQR 25.9-31.9) and 16.4%, 37.0% and 44.0% presented type 2 diabetes (T2D), high blood pressure and dyslipidemia, respectively. Human immunodeficiency virus (HIV) prevalence was 3.8% (12/320). Median liver stiffness was 5.2 kPa (4.2-6.4), 13.8% and 8.8% had LSM ≥ 8 and ≥ 10 kPa, respectively. We compared participants with LSM < 8 vs ≥ 8 kPa (Table 1) and no differences were found in terms of age, gender or ethnicity. Subjects with LSM ≥ 8 kPa showed higher rates of T2D (31.2% vs 14.1%, p 0.003), dyslipidemia (62.5% vs 41.1%, p 0.005) and obesity (58.3% vs 38.5%, p 0.009). HIV was more frequent in the LSM ≥ 8 kPa group (14.0% vs 2.2%, p < 0.001). In the multivariate analysis T2D (HR = 2.54; 95%CI 1.15-5.60, p 0.020), obesity (HR = 2.07; 95%CI 1.04-4.10, p 0.037) and HIV (HR = 5.82; 95%CI 1.67-20.3, p 0.006) were associated with high likelihood of significant liver fibrosis, contrary to older age and caucasian ethnicity.

Conclusion: Our preliminary results suggest that prison inmates suffer from a relevant metabolic burden, which along to other specific risk factors in this population, such as HIV, are associated with a remarkable prevalence of NAFLD significant fibrosis.

Figure:

Table 1: Characteristics according to the presence of significant liver stiffness measured by TE.

Variables	LSM \geq 8 kPa n=48	LSM < 8 kPa n=312	p value
Age, mean years (SD)	47.9 (13.3)	45.7 (12.2)	0.26
Male, n (%)	40 (83.3)	249 (79.8)	0.56
Caucasian, n (%)	33 (68.8)	184 (59.0)	0.19
BMI, median kg/m ² (IQR)	31.5 (27.7-35.6)	28.3 (25.7-31.5)	0.001
Obesity (\geq 30 kg/m ²), n (%)	28 (58.3)	119 (38.5)	0.009
Waist circumference, median cm (IQR)	106 (94-114)	98 (90-107)	0.001
Type 2 diabetes, n (%)	15 (31.2)	44 (14.1)	0.003
Arterial hypertension, n (%)	21 (43.8)	112 (36.0)	0.30
Dyslipidemia, n (%)	30 (62.5)	127 (41.1)	0.005
HIV, n (%)	6 (14.0)	6 (2.2)	<0.001
CAP, median dB/m (IQR)	288 (241-353)	244 (211-284)	<0.001
ELF, mean score (SD)*	9.48 (0.99)	9.25 (0.82)	0.24
PNPLA 3 G allele, n (%)**	5 (41.7)	17 (43.6)	0.90
TM6SF2 T allele, n (%)**	2 (16.7)	2 (5.1)	0.19

*ELF score available in 171 patients.

**Genetic polymorphisms study available in 51 patients.

BMI: Body mass index; HIV Human immunodeficiency virus; CAP: Controlled attenuation parameter, ELF: Enhanced liver fibrosis

P02-23 Comparison of survival rates as predicted by total tumor volume or tumor burden score in patients with hepatocellular carcinoma concurrent with fatty liver disease

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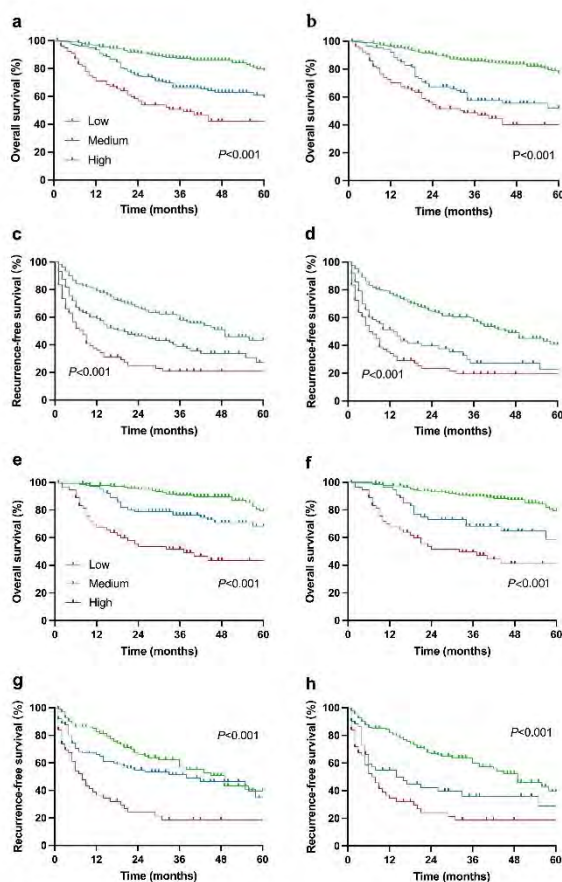
Background and aims: To compare the prognostic value of total tumour volume (TTV) and tumour burden score (TBS) in patients with hepatocellular carcinoma concurrent with fatty liver disease (FLD-HCC) or metabolic dysfunction-associated fatty liver disease (MAFLD-HCC) after hepatic resection.

Method: Patients diagnosed with FLD-HCC and treated with hepatic resection from January 2010 to December 2018 were retrospectively analyzed. The prognostic capabilities and predictive performance of TTV and TBS were determined. Overall and recurrence-free survival rates were compared using the Kaplan-Meier method, and independent risk factors were assessed using Cox regression analyses. Correlation analysis evaluated the relationship between TTV and TBS to estimate tumour burden.

Results: A total of 432 FLD-HCC patients were included in this study, of whom 301 were diagnosed with MAFLD-HCC. Overall survival was significantly higher among patients with low TTV or TBS than among those with medium or high TTV or TBS ($p < 0.001$). Higher TTV and TBS were independent risk factors for poor survival among patients with FLD-HCC or MAFLD-HCC ($p < 0.05$), whereas TTV showed compromised performance in predicting recurrence-free survival of MAFLD-HCC. TTV and TBS showed comparable discriminative ability in stratifying overall and recurrence-free survival of FLD-HCC or MAFLD-HCC patients, based on time-dependent receiver operating characteristic curves. Correlation analyses revealed a strong correlation between TTV and TBS in estimating tumour burden.

Conclusion: Both TTV and TBS may be effective predictors of overall survival in patients with FLD-HCC or MAFLD-HCC.

Figure: Kaplan-Meier analysis of overall and recurrence-free survival of patients with hepatocellular carcinoma concurrent with fatty liver disease, stratified by (a) and (c) total tumour volume (TTV) or (b) and (d) tumour burden score (TBS). Kaplan-Meier analysis of overall and recurrence-free survival of patients with hepatocellular carcinoma concurrent with metabolic dysfunction-associated fatty liver disease, stratified by (e) and (g) total tumour volume (TTV) or (f) and (h) tumour burden score (TBS).



P02-24 Boosting drug development in NASH through integrated research platforms: proposal of a master protocol for a NASH platform trial

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Abstract: Non-alcoholic fatty liver disease (NAFLD) is a condition that affects 25% of the population. Non-alcoholic steatohepatitis (NASH) is a progressive form of the disease that can lead to severe complications such as cirrhosis and hepatocellular carcinoma. Despite its high prevalence, no drugs are currently approved to treat NASH. The drug development pipeline in NASH is very active, yet most assets do not progress to phase 3 trials, and those that reach phase 3 often fail to achieve the end points necessary for approval by regulatory agencies. Amongst other reasons, the design and the methodological and operational features of traditional clinical trials in NASH might impede optimal drug development. Platform trials (PT) consist of multi-arm trials following a master protocol with a single control arm. Through interim analyses, these trials allow drug entry and early exit from the pipeline while providing participants better chances of receiving efficacious compounds. Master protocols often include periodic interim analyses using Bayesian algorithms for declaring futility or success using estimated posterior probabilities or frequentist methods that evaluate conditional power. Master protocols are often embedded in a clinical trial network; the master protocol, together with supporting research infrastructure, is known as an Integrated Research Platform (IRP). The EU Patient-centric clinical trial Platforms (EU-PEARL) project (IMI2-853966) aims at developing the necessary tools to deploy IRP in various diseases, including NASH. Altogether, PT represent an alternative for patients, pharmaceutical companies, and clinicians in the quest to approve a pharmacologic NASH treatment. In this work, the main design, statistical and regulatory challenges, and opportunities for a NASH IRP trial are discussed from various perspectives encompassing the views of patients, investigators, sponsors, and health authorities, along with the potential barriers that might be encountered owing to distinct and sometimes opposing priorities held by these stakeholders and potential ways to overcome them.

P02-25 A higher fibrosis-4 (FIB-4) score is associated with higher healthcare costs and hospitalizations in patients with non-alcoholic steatohepatitis

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Background and aims: The cost and complexity of care for patients with non-alcoholic steatohepatitis (NASH) increases with disease stage. We aimed to study the fibrosis-4 (FIB-4) score as a proxy for disease severity and test its association with increased disease burden.

Method: The US administrative database, Veradigm Health Insights Electronic Health Records linked with Komodo administrative claims data were used to identify adult patients with coded NASH who had aspartate aminotransferase (AST), alanine aminotransferase (ALT), platelet results, and age to compute FIB-4. The index date was the first coded NASH encounter between 2016 and 2020 with ≥ 6 months of database activity pre- and post-encounter and a FIB-4. Patients who were coded with viral hepatitis, alcoholism, or alcoholic liver disease were excluded from this analysis. Inpatient hospital admissions and log-transformed costs for pharmacy, hospital inpatient, emergency department, and outpatient services were measured in the 12-month period surrounding index. Multivariate logistic regression for any hospitalization and linear regression for log total healthcare cost was performed, controlling for patient demographics (age, race, sex, and geography), smoking status, Charlson comorbidity index (CCI), and diabetes complications severity index (DCSI).

Results: Overall, 6,743 patients met the study criteria. Mean age was 56.1 ± 13.3 years, 62.9% of patients were female, and 53.6% of patients were diagnosed with type 2 diabetes. Mean FIB-4 at index was 1.79 ± 1.88 . A 1-unit increase in FIB-4 at index was associated with a 4.2% increase in mean total annual healthcare costs ($p < 0.0001$; CI, 2.2% to 6.3%) and with an odds ratio of 1.12 ($p < 0.0001$; CI, 1.08 to 1.15) for hospitalization (Table). CCI and DCSI were also significantly associated with higher odds ratios for hospitalization (OR 1.27, $p < 0.001$; CI, 1.23 to 1.31 and OR 1.27, $p < 0.001$; CI, 1.22 to 1.33).

Conclusion: Higher FIB-4 score across a variety of ranges was associated with increased healthcare costs and hospitalizations in the NASH population.

Figure:**Table. Summary of modeling results for log total cost and hospital admissions**

Item	Log Total Cost			Hospital Admission		
	e ^b	e ^{Lower CI}	e ^{Upper CI}	Odds Ratio	Lower CI	Upper CI
FIB-4 at Index	1.042*	1.022	1.063	1.116*	1.080	1.153
Demographic Variables						
Age	0.997*	0.994	0.999	0.989*	0.983	0.994
Male	0.711*	0.662	0.763	0.891	0.784	1.013
White	0.905*	0.839	0.976	0.897	0.783	1.028
Hispanic	0.817*	0.732	0.912	0.876	0.715	1.073
Region: Northeast	1.384*	1.263	1.516	0.988	0.839	1.164
Region: Midwest	1.326*	1.185	1.484	1.072	0.881	1.305
Region: West	1.046	0.951	1.151	0.887	0.745	1.056
Region: Other	1.211*	1.046	1.402	1.181	0.921	1.515
Smoking Status						
Current Smoker	1.144*	1.029	1.272	1.089	0.902	1.315
Former Smoker	1.133*	1.018	1.262	1.093	0.903	1.323
Never Smoker	0.996	0.912	1.088	1.027	0.874	1.208
Health Status						
Charlson Comorbidity Index	1.276*	1.251	1.302	1.267*	1.225	1.310
Diabetes Complications Severity Index	1.232*	1.199	1.266	1.273*	1.219	1.329

*p<0.05

P03-01 Retrospective artificial intelligence-based measurement of NASH histology (AIM-NASH) analysis of biopsies from phase 2 study of resmetirom confirms significant treatment-induced changes in histologic features of non-alcoholic steatohepatitis

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Background and aims: Biopsy-based end points are recommended for assessing treatment efficacy in non-alcoholic steatohepatitis (NASH) clinical trials. While manual scoring of NASH histology is subject to intra- and inter-rater variability, artificial intelligence (AI)-powered pathology may enable accurate and reproducible histologic assessment. Here we demonstrate the utility of an AI-based tool (AIM-NASH) for scoring NASH histology in a retrospective analysis of liver biopsies.

Method: PathAI AIM-NASH algorithms were deployed on whole slide images of liver biopsies from 104 patients enrolled in a 36-week placebo-controlled Phase 2 trial (NCT02912260) which evaluated the safety and efficacy of resmetirom (MGL-3196), a liver-targeted, oral, once-daily thyroid hormone receptor-beta selective agonist, in patients with NASH and NASH Clinical Research Network (CRN) stage 1-3 fibrosis. Models predicted ordinal and continuous CRN scores in addition to exploratory features such as portal inflammation. Concordance between clinical pathology and AIM-NASH scores and features was evaluated. Differences in end point response rates and in changes in continuous scores and exploratory features between resmetirom-treated and placebo-treated patients were computed.

Results: All biopsy-based end points that were met via manual histologic scoring were also met by AIM-NASH (Table). Machine learning (ML) continuous scoring revealed a statistically significant reduction in steatosis and a greater sub-ordinal reduction in fibrosis in resmetirom-treated versus placebo-treated patients. The measured reduction in continuous fibrosis score was greater than the reduction in collagen proportionate area (CPA) in resmetirom-treated patients. Change in ML-derived area proportion of portal inflammation was significantly correlated with change in portal inflammation scores from both central readers ($p = 0.009$ and $p = 0.011$, respectively), while concordance between both central readers for portal inflammation scoring was kappa = 0.298.

Conclusion: This ML approach detected similar proportions of primary end point responders as manual scoring. ML-derived continuous features demonstrated sensitivity to changes in NASH histology and enabled more granular exploration of sub-ordinal treatment effects. Such AI-based tools show promise as assistive devices for NASH pathologists in clinical trial settings.

Figure:

Table. Response rates per endpoint via manual vs artificial intelligence-based scoring				
Endpoint	Scorer	Response rate (Resmetirom)	Response rate (Placebo)	P value
≥2-point improvement in NAS	AIM-NASH	0.41	0.19	0.0327
	Central reader	0.56	0.26	0.0044
	Reader 2	0.42	0.19	0.0321
NASH resolution without worsening of fibrosis	AIM-NASH	0.26	0.07	0.0301
	Central reader	0.25	0.06	0.0226
	Reader 2	0.21	0.03	0.0190
NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis.				

P03-02 Utility of FIB-4 thresholds to identify patients with at-risk F2-F3 non-alcoholic steatohepatitis based on screening data from a 2000 patient biopsy-confirmed cohort of resmetirom phase 3 trial (MAESTRO-NASH)

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Background and aims: MAESTRO-NASH (NCT03900429) is a 52-week randomized double-blind placebo-controlled Phase 3 registrational trial to evaluate the effect of resmetirom (MGL-3196), a liver-targeted, oral, once-daily thyroid hormone receptor-beta selective agonist, in patients with non-alcoholic steatohepatitis (NASH) and significant liver fibrosis. Eligibility requires ≥ 3 metabolic risk factors, FibroScan VCTE ≥ 8.5 kPa, and biopsy-confirmed NASH with fibrosis stage 1B, 2, or 3 (or 1A/C with PRO-C3 ≥ 14), and NAFLD activity score (NAS) ≥ 4 with ≥ 1 in each NAS component. FIB-4 ≥ 1.3 is recommended by various guidelines to identify potential at-risk NASH patients; patients with FIB-4 < 1.3 may be considered low risk.

Method: FIB-4 cutoff values of 1.3 and 1.0 were applied to approximately 2000 patients who screened for MAESTRO-NASH with screening labs, FibroScan, MRE, MRI-PDFF, and screening liver biopsy. Relationships between screening FIB-4 ≥ 1.0 and ≥ 1.3 and liver biopsy NAS and fibrosis stage were assessed.

Results: Overall, 56.9% of F2, 40.3% of F3, and 24.4% of F4 biopsy-confirmed patients had FIB-4 < 1.3 while 46.4% of patients with active NASH (NAS ≥ 4) fibrosis F2/F3 had FIB-4 < 1.3 (Table). 32.6% of F2 and 18.0% of F3 patients had FIB-4 < 1.0 . In patients with active NASH (NAS ≥ 4), 41.7% of F2 and 17.3% of F3 patients had FIB-4 < 1.0 . NAS ≥ 4 F2-F3 patients with FIB-4 ≥ 1.3 had mean age 61.1 years while NAS ≥ 4 F2-F3 patients with FIB-4 < 1.3 had mean age 52.2 years ($p < 0.001$); patients with FIB-4 ≥ 1.0 had mean age 59.9 years; NASH patients with FIB-4 < 1.0 had mean age 47.6 years ($p < 0.001$). More low-risk NAFLD patients (F0, F1A/C) had FIB-4 < 1.3 than FIB-4 < 1.0 (F0, 84.3% vs 58.1%, respectively). Absolute values of AST ($p < 0.0001$), ALT ($p < 0.0001$), PRO-C3 ($p < 0.0001$), HbA1c ($p = 0.0001$), GGT ($p < 0.0001$), and MRE ($p < 0.0001$) showed statistically significant differences between low-risk (F0) and high-risk (F2-F3) NASH patients and could be used to further stratify risk.

Conclusion: Based on a large Phase 3 data set of biopsy-confirmed NASH patients, FIB-4 ≥ 1.3 lacks the sensitivity to accurately identify patients with at-risk F2-F3 NASH. The influence of age on FIB-4 may require an age adjustment to ensure younger patients are not removed from consideration for therapy.

Figure:

Table.				
All Biopsy Patients	FIB-4 (1.3) n<1.3/Total Patients	%	FIB-4 (1.0) n<1.0/Total Patients	%
F0	199/236	84.3	137/236	58.1
F1A/C	212/293	72.4	147/293	50.2
F1B	119/184	64.7	74/184	40.2
F2	253/445	56.9	145/445	32.6
F3	303/752	40.3	135/752	18.0
F4	21/86	24.4	10/86	11.6
All Patients	1107/1996	55.5	648/1996	32.5
Patients with Eligible NAS ≥4	FIB-4 (1.3) %<1.3/NAS Eligible Patients		FIB-4 (1.0) %<1.0/NAS Eligible Patients	
F0	NA		NA	
F1A/C	58.9		28.6	
F1B	63.9		43.6	
F2	55.8		41.7	
F3	39.2		17.3	
F4	NA		NA	
All Patients	47.9		25.0	

P03-03-YI Fully connected neural network-based serum surface-enhanced Raman spectroscopy accurately identify non-alcoholic steatohepatitis

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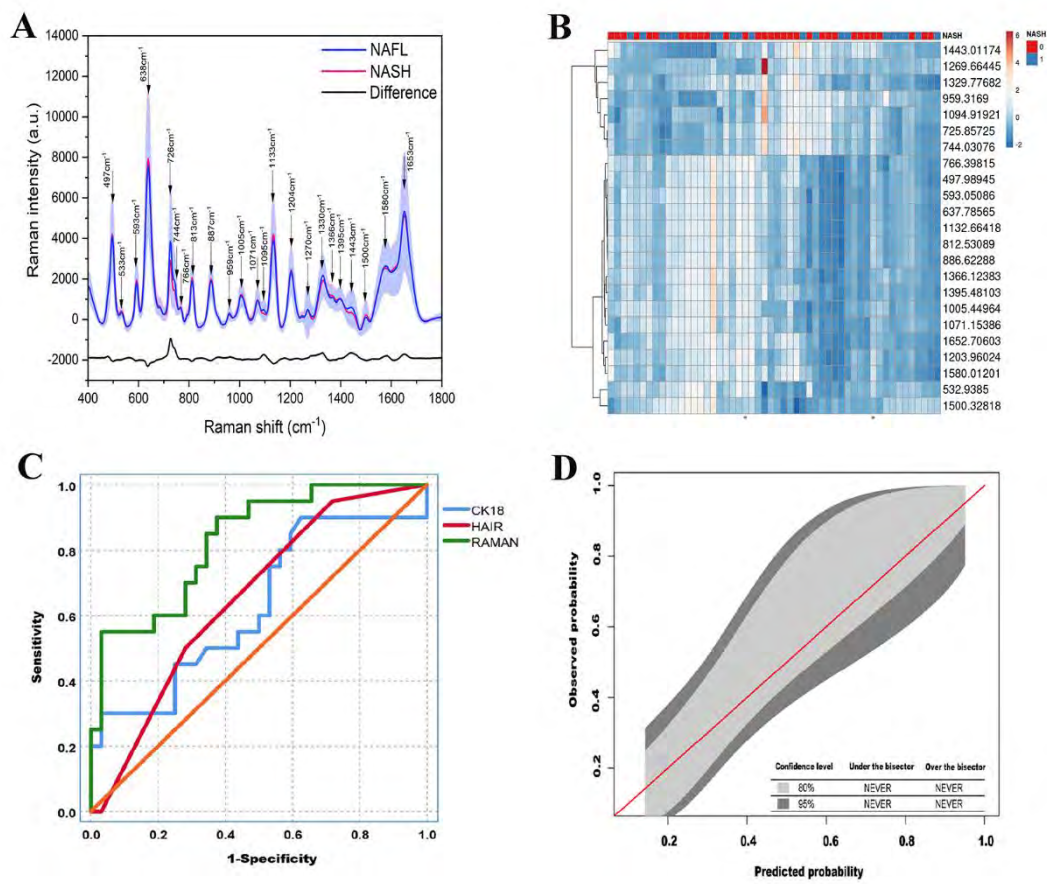
Background and aims: There is an increased need to find a standardized and low-risk diagnostic tool that can detect non-alcoholic steatohepatitis (NASH). Surface enhanced Raman spectroscopy (SERS), a technique combining Raman spectroscopy with nanotechnology, has recently received considerable attention due to its potential for improving medical diagnostics. We aimed to investigate combining SERS and neural network approaches, using a liver biopsy dataset to develop and validate a new diagnostic model for identifying NASH.

Method: Silver nanoparticles as the SERS-active nanostructures were mixed with blood plasma to enhance the Raman scattering signals. SERS spectra were normalized to the integrated area under the curve in the 400-1800cm⁻¹ wavenumber range after the removal of fluorescence background from the original SERS data. Then, the spectral data set was used to train the NASH classification model by a neural network consisting primarily of a fully connected residual module.

Results: Data on 261 patients with biopsy-proven NAFLD were included and a prediction model for NASH was built based on SERS spectra and neural network approaches. Fig. A compares the normalized mean SERS spectra obtained from NAFL subject blood serum samples and NASH patient serum samples. It can be seen that while significant SERS spectral differences exist between NAFL and NASH serum samples, primary SERS peaks at 497, 533, 593, 638, 726, 744, 766, 813, 887, 959, 1005, 1071, 1095, 1133, 1204, 1270, 1330, 1366, 1395, 1443, 1500, 1580, and 1653 cm⁻¹. Fig. B presents the association between the presence of NASH on liver histology and these selected SERS bands. The model yielded an AUROC of 0.83 (95% confidence interval [CI] 0.70-0.92) in the validation set, which was better than AUROCs of serum CK-18- M30 levels (AUROC 0.63, 95% CI 0.48-0.76, $p = 0.044$) and the HAIR score (AUROC 0.65, 95% CI 0.51-0.77, $p = 0.040$) (Fig. C). The calibration curve of the model showed good agreement between prediction and observation in the validation cohort (Fig. D).

Conclusion: Fully connected neural network-based serum SERS analysis provides a non-invasive, rapid and practical way for accurately identifying the presence of NASH.

Figure:



P03-05-YI Burden of hepatocellular cancer in patients with type-2 diabetes mellitus: a 2010-2020 national cohort study

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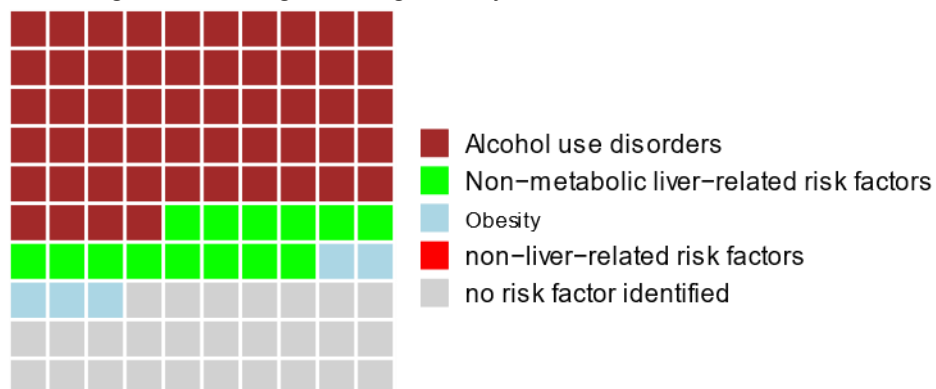
Background and aims: There are uncertainties on the burden of hepatocellular cancer (HCC) in patients with type-2 diabetes (T2D). We measured National incidences and risks for HCC and HCC-free mortality in patients with T2D.

Method: The data source was the 2011-2020 National French hospital discharge database. We selected all adults with T2D. The incidences of HCC and of HCC-free mortality were measured after 2013 to reduce the risk of recording non-incident HCC. Adjusted odds ratios (aOR) were computed with multinomial logistic regression models.

Results: Sample size was 2, 883, 684. Mean (IQR) age was 67 (58, 77) years and 54% were men. HCC was diagnosed in 25, 396 (0.9%) patients over 12, 516, 010 person-years [median (IQR) follow-up 4.4 (1.3-7.1) years]. HCC incidence (95% CI) was 1.49 (1.47-1.51) per 1000 person-years at risk. HCC incidence was maximum (>1.5% person-year) in patients aged 65-70 with alcohol use disorders. Attributable risks for alcohol use disorders, non-metabolic liver-related risk factors, and obesity were 54%, 14%, and 5%, respectively. In patients without a well-identified risk factors of liver disease progression, HCC incidence was 0.59 (0.58-0.61) per 1000 person-years at risk. Male sex, age between 60-70 years, alcohol use disorders [aOR 17.9 (17.3-18.5)], non-metabolic liver-related risk factors [aOR 8.1 (7.7-8.4)] and obesity [aOR 1.09 (1.06-1.13)] were independently associated with a higher risk of HCC than of HCC-free mortality. Deprivation was not associated with HCC.

Conclusion: Alcohol use disorders was the main driver of liver disease progression to HCC in patients with T2D in France 2011-2020. Patients with alcohol use disorders, non-metabolic liver-related, or obesity were, *ceteris paribus*, at higher risk of HCC than of HCC-free mortality. Patients with T2D should be advised to drink minimal amounts of alcohol.

Figure: The attributable fraction reflects the number of HCC that would have been prevented in the absence of a risk factor. Attributable fractions were computed with binary logistic regressions adjusted for sex, age at censoring, smoking, obesity, liver-related and non-liver-related risk factors.



P03-06-YI Echocardiography-based markers of subclinical cardiac dysfunction in individuals with non-alcoholic fatty liver disease and preserved ejection fraction

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Background and aims: Individuals with Non-Alcoholic Fatty Liver Disease (NAFLD) have abnormal myocardial energy metabolism and reduced coronary functional capacity, even in the absence of risk factors for cardiovascular disease (CVD), potentially associated with cardiac fibrosis and heart failure (HF). We aimed to evaluate diastolic and systolic function in NAFLD individuals with preserved ejection fraction (EF).

Method: We prospectively included patients with ultrasound-diagnosed NAFLD undergoing screening echocardiography per protocol, in the absence of overt CVD or HF. Echocardiography was performed according to European Society of Cardiovascular Imaging guidelines, including speckle tracking analysis with left ventricular global longitudinal strain (GLS) measurement for accurate quantification of systolic function (Philips, Andover, US). Diastolic dysfunction with increased filling pressures was defined as a mitral E/E' ratio >9. Liver fibrosis was assessed by non-invasive tests and transient elastography (TE, Fibroscan F530); significant fibrosis was defined by either FIB-4 score >1.3 or liver stiffness (LS) >7 kPa. Clinical and biochemical parameters, as well as TE and echocardiography, were collected within one month from NAFLD diagnosis.

Results: A total of 56 patients were included. Median age was 53.5 [IQR 46.5-61.0] years and 57.1% was male. Type 2 diabetes mellitus (T2DM) and obesity were present in 20.4% and 40.7% of the cohort, while 45.5% had arterial hypertension. Median FIB-4 was 0.96 [IQR 0.62-1.23]. Median LS was 5.2 [IQR 4.6-5.9] kPa and significant fibrosis was present in 12.5% of the total. Median EF was 62.0% [IQR 60.0-64.8]. Median E/E' was 7.0 [IQR 5.5-8.0] and E/E' ratio >9 was present in 21.8% of the total. Median GLS was -19.7% [-21.0%, -18.6%]. Mitral E/E' was significantly higher in individuals with hypertension ($p = 0.017$) and T2DM ($p = 0.0009$). Individuals with LS >7 kPa had higher values of E/E' ($p = 0.018$) and decreased GLS values (median -18.3% versus -19.9% of the group with LS ≤7 kPa, $p = 0.032$). FIB-4 was significantly higher in individuals with diastolic dysfunction ($p = 0.025$). LS and FIB-4 positively correlated with E/E' ($r = 0.33$, $p = 0.015$ and $r = 0.42$, $p = 0.005$, respectively). In a multivariate logistic regression model including significant fibrosis, T2DM and hypertension, only T2DM was associated with E/E' >9 (adjusted OR 10.6 [95%CI 1.1-99.2], $p = 0.040$).

Conclusion: In NAFLD patients with preserved EF, significant liver fibrosis by FIB-4 and TE correlate with markers of diastolic dysfunction and lower GLS systolic values. T2DM is the strongest factor associated with diastolic dysfunction in this population.

The research has been supported by the Italian Ministry for Education, University and Research (MIUR) under the programme "Dipartimenti di Eccellenza 2018-2022" Project code D15D18000410001.

P03-08 Pregnancy as a unique opportunity to identify NAFLD in women: a prospective assessment

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) in U.S. women of reproductive age is rising and recent administrative claims-based data suggest a significant association of NAFLD with adverse pregnancy outcomes. We evaluated the prevalence and associated risk factors of NAFLD at a high-volume obstetrics center.

Method: In this prospective study, a liver ultrasound at time of routine pregnancy anatomy scan at 18-22 weeks' gestation was performed to assess for presence of NAFLD. Obstetric sonographers were trained to obtain liver images that were subsequently graded for hepatic steatosis by a radiologist blinded to clinical status. The proportion having elevated hepatic steatosis index (HSI) and meeting recently defined metabolic associated fatty liver disease (MAFLD) criteria was evaluated. A multivariable logistic regression model assessed independent predictors of NAFLD among pregnant women.

Results: Among 749 women approached for participation, satisfactory liver ultrasounds were obtained on 560 (75%) pregnant individuals; median age 28, 58% Hispanic ethnicity, 65% with pre-pregnancy BMI ≥ 25 , 22% nulliparous. Seventy-eight (14.3%) had steatosis on ultrasound; 83% grade 1, 14% grade 2 and 3% grade 3. Overall, 36% had HSI ≥ 36 ; 11% satisfied MAFLD criteria. Only 4 of 560 (0.71%) carried a previous diagnosis of NAFLD. Women with steatosis were more likely to be of Hispanic ethnicity, have diagnoses of chronic hypertension and diabetes and have a pregnancy history of gestational diabetes ($p < 0.05$; see Table). Women with grade 2/3 steatosis were more likely to have a history of preeclampsia (30% vs 10%, $p = 0.05$). In multivariable analyses, Hispanic ethnicity (OR 2.57; 95% CI 1.30-5.09) and BMI (OR 1.06; 95% CI 1.01-1.11) were independently associated with NAFLD.

Conclusion: In this first time U.S. based prospective study of pregnant women, integration of ultrasound into obstetric care was feasible and effective in identifying women with NAFLD. NAFLD prevalence was 14%, similar to reports in non-pregnant women of reproductive age with Hispanic and elevated pre-pregnancy BMI women being at highest risk of NAFLD. Most had no prior diagnosis of NAFLD, highlighting a unique opportunity to identify and link women to specialized care.

Figure:

	No NAFLD n = 469 (85.7%)	NAFLD n = 78 (14.3%)	p value
Age (median, IQR)	28 (24, 33)	28 (24, 33)	0.666
Hispanic ethnicity, n (%)	254 (56%)	60 (77%)	0.002
Pre-pregnancy BMI (kg/m ²), median	26 (23, 31)	29 (25, 34)	0.001
Chronic HTN, n (%)	31 (7%)	11 (14%)	0.022
Type II DM, n (%)	9 (2%)	7 (9%)	0.001
Autoimmune disease, n (%)	23 (5%)	8 (11%)	0.055
Prior Pregnancy History			
Gestational Diabetes	17 (5%)	7 (13%)	0.023
Preeclampsia	34 (10%)	10 (18%)	0.065
Cholestasis of Pregnancy	7 (1%)	0 (0%)	0.291
Preterm birth	53 (15%)	6 (11%)	0.395
NAFLD Clinical scores			
Mean HSI (SD) (n = 368)	37.88 (8.44)	40.84 (8.97)	0.013
No. Satisfying <u>any</u> MAFLD Criteria (%) (n = 549)	4 (0.85)	58 (74.36)	0.001

P03-10 Impact of non-alcoholic fatty liver disease (NAFLD) on in-patient outcomes in non-variceal upper gastrointestinal bleeding: a nationwide analysis

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Background and aims: NAFLD is slowly becoming the most common liver disease in the world. It includes a wide spectrum of liver diseases from fatty infiltration, fibrosis, to cirrhosis. Upper GI bleeding has been classified into variceal or non-variceal bleeding. Although variceal bleeding is commonly seen in chronic liver disease, at least 30-40% of liver disease population may have nonvariceal upper GI bleeding. We aim to evaluate the clinical outcomes and predictors of mortality in patients with NAFLD who present with non-variceal upper GI bleeding.

Method: Using de-identified data from the National Inpatient Sample (NIS) database 2016-2019, we identified patients with non-variceal UGIB and then stratified them into those with and without NAFLD. Patient demographics, length of stay, hospital charges, comorbidities, complications and mortality outcome data were analyzed. Mann-Whitney tests with Bonferroni corrections were used for testing differences in continuous variables, while chi-squared tests with Bonferroni corrections were used for testing homogeneity of categorical variables. Multivariate logistic regression was conducted to analyze the relationship between mortality and NAFLD, while controlling for relevant covariates. Bidirectional stepwise regression was utilized to build the final model. All statistical analysis and hypothesis tests were performed at significance level, with p value set at <0.05. Analyses were conducted using R software (v. 4.0.4).

Results: Multivariate logistic regression analysis (MLRA) was conducted, controlling for the multiple covariates. The primary outcome of interest, mortality, was found to be significantly higher in patients with NAFLD and GI bleeding [aOR: 1.88 (1.68-2.1)]. Secondary outcomes of interest, shock [aOR: 1.15 (1.07-1.22)], acute respiratory failure [aOR:1.28 (1.15-1.42)] and acute liver failure [aOR:5.58 (4.48-6.69)] were all more likely to occur in this cohort. Patients with NAFLD were also more likely to incur higher hospital charges [\$2174 (\$1677-\$2618)] and have a longer length of stay [0.28 days (0.17-0.38)]. Interestingly, in our study the patients with NAFLD were less likely to suffer from acute myocardial infarction [aOR: 0.73 (0.64-0.85)]. Patients with NAFLD were not more likely to suffer AKI, shock requiring vasopressors, sepsis, blood transfusion, intubation or dialysis.

Conclusion: Our analysis showed that patients with non-variceal UGIB have higher mortality, increased complications, longer length of stay and higher hospital charges pointing to the increased morbidity and economic burden of NAFLD.

Figure:

Figure 1: Comparison of demographic and co-morbidities data between non-variceal UGIB with and without NAFLD.

Figure 2: Forest Plot showing relation between presence of NAFLD with GI bleeding and co-variates.

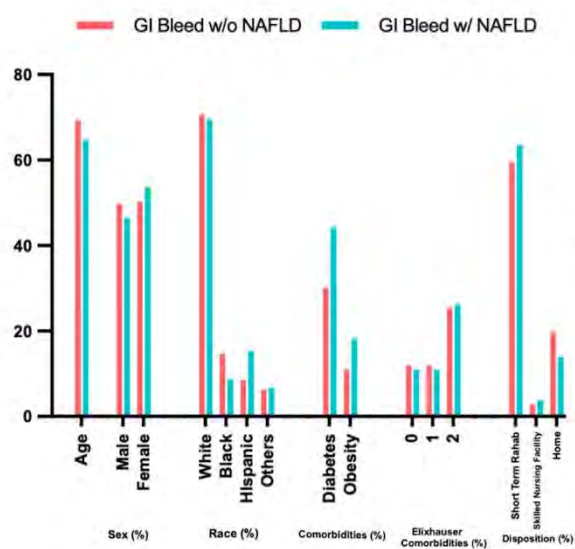


Figure. 1

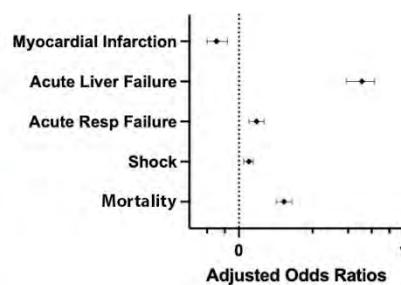


Figure. 2

P03-11 NAFLD-related liver fibrosis is associated with impaired bone mineralization and degraded micro-architecture in obesity

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Background and aims: Chronic liver diseases are associated with increased bone fracture risk, mostly in end-stage disease and cirrhosis; besides, evidence on the relationship between non-alcoholic fatty liver disease (NAFLD) and bone fragility is limited and no data are available on NAFLD/NAFLD-related fibrosis and bone microarchitecture impairment. Aim of this study was to investigate parameters of bone mineralization and microstructure in obese individuals with NAFLD in relation to estimated liver fibrosis.

Method: For this cross-sectional investigation, we analyzed data from 1872 obese individuals (44.6 ± 14.1 years, male/female: 389/1483, BMI: 38.3 ± 5.3 kg/m²) referring to the Endocrinology outpatient clinics of Sapienza University, Rome, Italy. Participants underwent clinical work-up, blood sampling for metabolic profiling and Dual-Energy X-ray Absorptiometry (DXA) for measuring the bone mineral density (BMD) and the trabecular bone score (TBS) as index of bone microarchitecture. NAFLD was diagnosed and quantified by Fatty Liver Index (FLI) and Liver Fat Score (LFS); liver fibrosis was estimated by FIB-4. Serum PTH, 25 (OH) vitamin D, calcium, phosphate and osteocalcin levels were also measured.

Results: Individuals with osteopenia/osteoporosis (T score < -1) had significantly greater FIB-4 than those with normal BMD (mean \pm FIB-4: 1.01 ± 0.71 vs 0.75 ± 0.44 ; $p < 0.001$). FIB-4 progressively increased in presence of partially to markedly degraded bone microarchitecture ($p < 0.001$) and negatively correlated with serum osteocalcin ($r = -0.17$, $p < 0.001$). Higher FIB-4 predicted bone fragility with OR 3.8 (IC95% 1.5- 9.3); this association persisted statistically significant after adjustment for sex, age, BMI, T2DM, smoking status and PTH at the multivariable logistic regression analysis (OR 1.91 (IC95% 1.15-3.17), $p < 0.01$), with adjusted AUROC = 0.842 (95% C.I.: 0.795-0.890; $p < 0.001$).

Conclusion: Our data indicate the presence of a tight relation between NAFLD-related liver fibrosis, lower bone mineral density and degraded microarchitecture in obese individuals, suggesting potential common pathways underlying liver and bone involvement in insulin-resistance associated disorders.

P03-12-YI Diagnosis of NAFLD with and without fibrosis using a lower ALT reference range in the iLFT system

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Background and aims: Up to 30% of people with non-alcoholic fatty liver disease (NAFLD) are at risk of cirrhosis and subsequent decompensation, making it crucial to identify them early. Using the conventional normal ALT range (≥ 55 U/L), rather than the true healthy normal range (≥ 30 U/L), risks missing many potential NAFLD cases. Intelligent liver function testing (iLFT) uses minimal diagnostic criteria and non-invasive fibrosis scores to automatically investigate abnormal LFTs, generating diagnoses and management plans in primary care. This saved costs and increased liver disease diagnoses by 44% using the conventional ALT range. As part of a secondary project, iLFT also triggered cascades for patients with ALT ≥ 30 U/L. This study investigates the impact of this on NAFLD diagnosis.

Method: A retrospective analysis was performed to identify all NAFLD diagnoses with and without abnormal NAFLD Fibrosis Score or Fib-4 score detected by iLFT between 2016-22. Patients were stratified into three groups: ALT 30-41 U/L, ALT 42-54 U/L and ALT ≥ 55 U/L. Clinical records were reviewed to determine if patients were referred to liver clinics, if Enhanced Liver Fibrosis (ELF) score or transient elastography (TE) was performed, and the final diagnosis.

Results: 16, 373 cases were identified in total. iLFT detected 986 NAFLD cases with normal fibrosis scores. 211 (21%) had ALT 30-41 U/L, 196 (20%) had ALT 42-54 U/L, and 509 (52%) had ALT ≥ 55 U/L. These patients were managed in primary care.

iLFT detected 871 NAFLD cases with indeterminate or high risk for advanced fibrosis scores: 139 (16%) had ALT 30-41 U/L; 137 (16%) had ALT 42-54 U/L; and 493 (57%) had ALT ≥ 55 U/L. 576 (66%) were referred to liver clinics. Of those, 111 (19%) patients had ELF scores suggesting low risk of advanced fibrosis (<9.8) and were not seen. 173 (30%) patients had a final diagnosis of NAFLD with evidence of fibrosis; 25 (15%) had ALT 30-41 U/L; 32 (19%) had ALT 42-54 U/L; and 116 (67%) had ALT ≥ 55 U/L. Of those, 123 (71%) had moderate-severe fibrosis on TE or clinical decompensation. 17 (14%) had ALT 30-41 U/L; 23 (19%) had ALT 42-54 U/L; and 74 (60%) had ALT ≥ 50 U/L.

171 (37%) patients had a diagnosis of NAFLD without fibrosis, and 76 (16%) patients had a non-NAFLD diagnosis.

Conclusion: A substantial proportion (33%) of NAFLD patients with fibrosis had ALT between 30-54 U/L. This meant that iLFT referred 57 patients for further assessment who would otherwise have been missed using the conventional ALT range. This suggests we should consider lowering the reference range for ALT to improve the diagnosis of NAFLD in primary care.

Figure:

Table 1: Number of iLFT and clinic diagnoses of NAFLD patients at different ALT thresholds.			
	ALT 30-41	ALT 42-54	ALT ≥55
iLFT diagnosis:			
NAFLD with normal fibrosis scores	211	196	509
NAFLD with indeterminate/high risk fibrosis scores	139	137	439
Clinic diagnosis:			
NAFLD with fibrosis	25	32	116
NAFLD without fibrosis	32	29	110
Non-NAFLD	9	11	56

P03-15 Glomerular hyperfiltration is a marker of cardiovascular damage and fibrosis severity of non-alcoholic fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) represents a risk factor for the development of cardiovascular disease (CV) and chronic kidney failure (CKD), early expressed by glomerular hyperfiltration. However, the association between glomerular filtrate, different degrees of NAFLD and CV risk is not described in the literature.

Method: 133 patients with NAFLD diagnosed by abdominal ultrasound and Fibroscan CAP, were enrolled. The degree of steatosis and stiffness in all subjects were assessed by Fibroscan echoSense with CAP module. Glomerular filtration rate (eGFR) was estimated using the 2021CKD-EPI formula. Two different classes were defined on the basis of filtrate: normal (GFR: <110 and >60 ml/min) (nGFR) and hyperfiltration (GFR \geq 110 ml/min) (hGFR). For CV risk, echocardiogram and Pulse Wave velocity (PWV) were performed. Main anthropometric and biochemical indexes are recorded.

Results: Of the 133 enrolled patients, 111 were in nGFR group while 22 in hGFR group. Mean age was 53 years \pm 12 SD. The two groups did not show statistically significant differences for age, sex, arterial blood pressure or body mass index. On the other hand, the hGFR group was characterized by a worse cardiovascular and hepatic profile compared to nGFR. In particular, the hGFR group showed at echocardiographic evaluation a concentric remodeling with a Left Ventricular Mass index (LVMI) values of 90.1 g/m² compared with the nGFR (LVMI of 75 g/m²) ($p < 0.006$). With regard to CV assessment, hGFR patients had a mean PWV of 8.5 m/sec, compared with nGFR with a mean PWV of 7.7 m/sec ($p < 0.05$). Regarding liver profile, in hGFR group the mean stiffness and steatosis grade were 7.3 kPa and 1.8 point, respectively, compared to nGFR (5.8 kPa and 1.5 point), respectively ($p < 0.05$).

Conclusion: The group of patients with glomerular hyperfiltration (hGFR) was characterized by a worse in CV index and liver profile. Therefore, glomerular hyperfiltration could represent an early marker of liver damage/fibrosis, useful to detect it and increased CV risk in patients with NAFLD.

P03-16 Secretome analysis of non-diabetic patients with non-alcoholic fatty liver disease identifies glucose-dependent insulintropic peptide (GIP) and interferon gamma-induced protein 10 (IP-10) as biomarkers of “at risk non-alcoholic steatohepatitis”

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Background and aims: Non-Alcoholic Fatty Liver Disease (NAFLD) encompasses a wide spectrum of clinical conditions ranging from simple steatosis to Non-Alcoholic SteatoHepatitis (NASH). Patients with active NASH and significant liver fibrosis are those at higher risk of progression to liver cirrhosis. We performed a serum secretome analysis in non-diabetic NAFLD patients to identify biomarkers of “at risk NASH”.

Method: A total of 149 biopsy-proven non-diabetic NAFLD patients (median age: 44, 34-52 years; 124 males and 81 females; median body mass index [BMI]: 29.0, 26.1-33.1 kg/m²; median alanine aminotransferase [ALT]: 48, 31-77 IU/ml) were included in the study. Patients with NASH + NAFLD Activity Score ≥ 4 + liver fibrosis stage (F) ≥ 2 were defined as “at risk NASH”. Secretome analysis was performed by Bio-Plex Multiplex Immunoassays (48-Plex-12007283 and 10-Plex-171A7001M panels, BioRad Laboratories, Hercules, CA, USA). Diagnostic accuracy was assessed by receiver operating characteristics curve analysis and reported as area under the curve (AUC).

Results: Overall, 107 (71.8%) patients had a diagnosis of NASH; 46 (30.9%) were F ≥ 2 . A total of 23 (15.4%) patients met the definition of “at risk NASH”. We found that several cytokines (IL-18 and IL-2R α), chemokines (IP-10, MIG, and SCDF-1 α), adipokines (visfatin and resistin), hormones (insulin, glucagon, leptin, GIP, and GLP-1), and related proteins (c-peptide) were significantly increased in patients “at risk NASH”. By multivariate regression analysis adjusted for age, BMI and ALT, only GIP and IP-10 resulted significantly and independently associated to “at risk NASH” (OR = 1.008, 95% CI 1.005-1.012, p <0.001 and OR = 1.001, 95% CI 1.000-1.003, p = 0.014, respectively). The corresponding accuracies for the detection of patients “at risk NASH” were 0.778 for GIP and 0.722 for IP-10. The combination of GIP + IP-10 further improved the performance for the identification of patients “at risk NASH” (AUC = 0.844).

Conclusion: Serum secretome analysis revealed a specific protein secretion profile associated to active NASH and significant fibrosis in non-diabetic NAFLD patients. In particular, the combination of GIP + IP-10 may serve as potential biomarker for the identification of patients “at risk NASH”.

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P03-17-YI A calorie-unrestricted low carbohydrate high fat diet improves non-alcoholic fatty liver disease (NAFLD) activity score (NAS) and HbA1c in type 2 diabetes: a six-month randomised controlled trial

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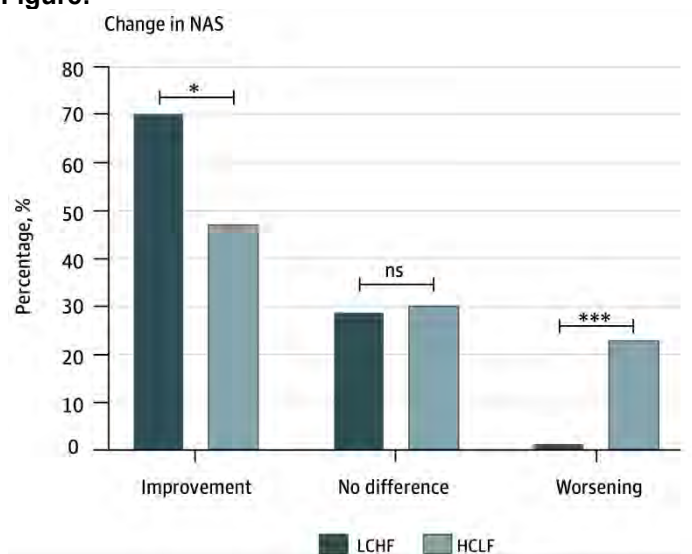
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Background and aims: NAFLD affects 55% of people with type 2 diabetes mellitus (T2DM), and glycaemic control predicts the severity of ballooning and fibrosis in NAFLD. Dietary interventions with low carbohydrates improve glycaemic control but the effect on NAFLD activity is unknown. We aimed to investigate the effect of a six-month low-carbohydrate high fat (LCHF) diet on NAFLD assessed by ≥ 2 points improvement in the NAFLD Activity Score (NAS) with at least 1 point improvement in either lobular inflammation or ballooning without worsening of fibrosis and on glycaemic control.

Method: We conducted a six-month randomised controlled diet trial in 185 people with T2DM. Participants were randomised 2:1 to LCHF or to a diet consisting of high carbohydrates and low fat (HCLF). Both diets were calorie-unrestricted. The LCHF diet consisted of maximum 20 energy percent (E%) carbohydrates, 50-60E% fats and 25-30E% proteins. The HCLF diet consisted of 50-60E% carbohydrates, 20-30E% fats and 20-25E% proteins. We performed liver biopsies and measured HbA1c (mmol/mol) at baseline and after six months. Biopsies were scored in a blinded matter according to the Non-alcoholic Steatohepatitis Clinical Research Network. The participants had ongoing dietitian consultations and compliance was reported continuously through an online food diary platform.

Results: Out of 185 randomised participants, 165 commenced the allocated intervention and were included in the analysis. At baseline the mean age was 56 (SD, 10) years, 58% were female, 88% had NAFLD, median NAS was 3 (1-5) and mean HbA1c was 56 (SD, 10) mmol/mol. After intervention we saw no significant difference between the groups in relation to improvement of ≥ 2 points in NAS ($p = 0.587$). However, more participants in the LCHF group improved NAS with ≥ 1 point compared to the HCLF group (70% versus 49%; $P = 0.028$), and fewer in the LCHF group experienced a worsening of NAS compared to the HCLF group (1% versus 23%; $P < 0.001$) (Figure). Participants in the LCHF group improved HbA1c with -9.5mmol/mol versus -3.4mmol/mol in the HCLF group ($p < 0.001$) and lost significantly more weight than in the HCLF group (-5.7kg vs. -1.8kg; $P < 0.001$). The self-reported macronutrient intake in LCHF versus HCLF throughout the intervention was 13/46E% carbohydrates, 61/29E% fats and 23/21E% proteins.

Conclusion: A six-month calorie-unrestricted LCHF diet improves NAS and HbA1c significantly more than a HCLF diet in people with T2DM.

Figure:

P03-18-YI Prognostic value of liver stiffness and FIB-4 score in individuals with cirrhosis due to non-alcoholic fatty liver disease without previous liver decompensation

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Background and aims: Liver cirrhosis due to Non-Alcoholic Fatty Liver Disease (NAFLD) represents a relevant cause of morbidity and mortality worldwide. Non-invasive tools are highly required in clinical practice to help physicians predict the incidence of hard outcomes in compensated cirrhotic individuals. The aim of this study was to explore the prognostic value of liver stiffness (LS) and FIB-4 score in individuals with NAFLD-related cirrhosis without previous liver decompensation.

Method: Individuals with NAFLD-cirrhosis were retrospectively selected for the absence of liver decompensation and a minimum follow-up of 6 months at the GI Division of the University of Torino. Diagnosis of NAFLD-cirrhosis was made by either liver histology or instrumental findings. Clinical and biochemical data were collected at the time of diagnosis. LS was assessed by transient elastography (Fibroscan) within one month from diagnosis. Follow-up events were collected for each patient and included: liver related events (ascites, encephalopathy, variceal bleeding), hepatocarcinoma (HCC), non-liver related events (cardiovascular events and extra-hepatic cancers), death and liver transplant.

Results: A total of 96 cirrhotic patients were included. Median age was 61.0 [IQR 51.0-67.0] years and 53.1% was male. Overall, 55.2 % patients had type 2 diabetes and 60.2 % were obese according to Body Mass Index. Median LS was 21.3 kPa [IQR 16.3-28.3] and median FIB-4 was 2.50 [IQR 1.73-4.13]. Median follow-up was 74 [IQR 44.6-135.0] months. Clinical events occurred in 39 (40.6%) patients, of which 22.9 % were not liver-related, while 21.9 % were liver-related (18.8% ascites, 12.5% encephalopathy, 5.2 % esophageal bleeding). HCC arose in 10.4 % of the total. Overall, 13 patients (13.5%) died and 3 (3.1 %) underwent liver transplant. Patients with LS >25 kPa had significantly increased incidence of liver-related events when compared to those with LS ≤25 kPa (median liver event-free survival 125.7 [99.1-152.4] versus 174.2 [162.2-186.2] months, logrank $p = 0.043$). FIB-4 >2.94 (cut-off by Youden Index with Se 100 %, Sp 68.9 % and Area Under the Curve of 0.79) was associated with increased incidence of overall mortality (HR 8.1 [95 % CI 2.2-30.8], $p = 0.001$).

Conclusion: Among individuals with NAFLD-related cirrhosis without previous liver decompensation, LS >25 kPa score was associated with increased incidence of liver-related events and FIB-4 >2.94 was associated with increased incidence of overall mortality.

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P03-19 Impact of intermittent fasting on anthropometric and clinical outcomes in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis

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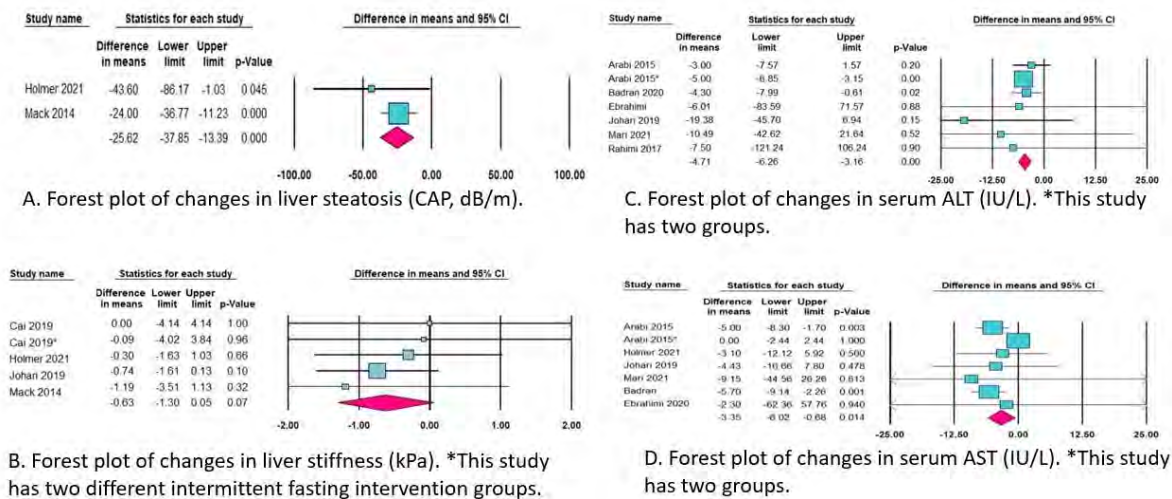
Background and aims: Currently, weight loss through caloric restriction is the cornerstone of initial non-alcoholic fatty liver disease (NAFLD) management. However, there remains a lack of evidence-based guidelines on the benefits of intermittent fasting (IF) for NAFLD. In this systematic review with meta-analysis, we evaluated the effect of IF on anthropometric and clinical outcomes in adults with NAFLD.

Method: We conducted a comprehensive search of MEDLINE, EMBASE, and Cochrane Central databases as well as trial registries and conference abstracts up to January 31, 2022. The search strategy included all appropriate controlled vocabulary and keywords for NAFLD and fasting. No date, language, or article type restrictions were included in the search strategy. Studies of adults with NAFLD or non-alcoholic steatohepatitis (NASH) undergoing an intermittent fasting type intervention reporting at least one anthropometric or clinical outcome were included in our review. A random effects model was used for meta-analysis to estimate mean differences between intervention and control group for the various outcomes.

Results: Initial literature search yielded 16 294 studies, of which 10 772 were duplicates, leaving 5517 studies to screen for title/abstract. An additional 5367 studies were excluded after title/abstract review with moderate concordance ($k = 0.53$). The remaining 150 studies were assessed for full text review with fair concordance ($k = 0.24$). Twelve studies were included in the systemic review and meta-analysis, totaling 908 participants with NAFLD, combined mean age of 42.50 years (SD 14.54), 50% male. Meta-analysis of anthropometric outcomes showed that BMI decreased significantly by -0.72 kg/m^2 (95% CI -1.07 to -0.37 , $p < 0.001$) following IF with no significant heterogeneity ($I^2 = 12.2\%$, $p = 0.33$). IF did not impact body weight (-2.40 kg , 95%CI -5.17 to 0.37 , $p = 0.09$), though between-study heterogeneity was substantial and significant ($I^2 = 83.5\%$, $p < 0.01$). IF significantly reduced liver steatosis, serum ALT, and serum AST in comparison to controls (CAP: -25.62 dB/m , 95% CI: -37.9 to -13.4 , $p < 0.001$; ALT: -4.71 IU/L , 95% CI: -6.26 to -3.16 , $p < 0.001$; AST: -3.35 IU/L , 95% CI: -6.02 to -0.68 , $p < 0.05$). However, liver stiffness was not significantly impacted (-0.63 kPa , 95% CI: -1.3 to 0.05 , $p = 0.07$). Heterogeneity for all clinical outcomes was not significant and below 40%.

Conclusion: Intermittent fasting interventions likely provide weight loss and hepatic benefits in adults with NAFLD. Studies with varying types of intermittent fasting interventions and intervention durations likely contributed to meta-analysis heterogeneity. Future randomized controlled studies of longer durations are needed to further validate the use of IF for NAFLD treatment.

Figure:



P03-20 Hepatocellular cancer surveillance in cirrhotic patients with fatty liver disease

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Background and aims: Surveillance for hepatocellular cancer (HCC) with six monthly ultrasound (US) is currently recommended for patients with cirrhosis. Due to factors such as obesity it is uncertain whether the benefit of US surveillance translates to patients with cirrhosis secondary to non-alcoholic fatty liver disease (NAFLD). We aimed to determine whether US surveillance in patients with NAFLD cirrhosis is effective in detecting early HCC.

Method: A retrospective cohort study of NAFLD patients with cirrhosis was performed at a tertiary Western Australian hospital between 2004 and 2022. Patients were identified from a hospital NAFLD database with cirrhosis confirmed by histology or radiological features. Patients with a prior history of HCC or with secondary causes of liver disease (e.g alcohol or viral) were excluded. HCC was diagnosed by histopathology or contrast enhanced imaging (as per AASLD guidelines). The absence of HCC was defined as no lesion on contrast enhanced cross sectional imaging or on follow-up US 12 months later. Participation in a surveillance program was defined as 6 (\pm 2) monthly ultrasounds occurring at least 70% of the follow-up period.

Results: 101 patients (53 females) with a mean age of 62.8 (\pm 14.1) years were included. 68 patients underwent surveillance for a median of 3.3 (0.5-12.2) years. The median MELD score of those on a surveillance program was equivalent to those not [8 (6-27) vs 8 (6-30, p = .45]. 32 patients were diagnosed with HCC; those on a surveillance program (n = 13) compared to those not (n = 19), were diagnosed at an earlier stage (BCLA 0-A) (85% vs 47%, p = .03), and with smaller lesions (24.4mm vs 46.6mm, p < .05). The one year cumulative incidence of HCC in the surveillance cohort was 6.1% (95% CI: 2.3-15.5%). Of the HCC patients who were on a surveillance program; 6 were detected from the program (46%), 4 detected on interval imaging performed for clinical indications (31%) and 3 on explant liver (23%). The median (range) BMI was similar for patients with HCC that was detected or missed in the surveillance program (31.2 (26.3- 41.8) kg/m² vs 32.5 (20.4- 54.6) kg/m² respectively, p = .4). Of the 19 patients with HCC diagnosed not during a surveillance program, 17 (89%) were referred as a new diagnosis of NAFLD cirrhosis with HCC. The sensitivity and specificity of surveillance detecting HCC >10mm was 50% and 69% respectively, and for HCC >20mm it was 75% and 91% respectively.

Conclusion: Patients with cirrhosis from NAFLD on regular US surveillance programs had HCC detected at earlier stages than patients not on surveillance. BMI was not associated with the rate of HCC detection by US.

Figure:

Barcelona stage	HCC detected on surveillance	HCC missed on surveillance	HCC detected not on surveillance
0	2 (33%)	2 (29%)	2 (10%)
A	3 (50%)	4 (57%)	7 (37%)
B	1 (17%)	1 (14%)	6 (32%)
C	0	0	3 (16%)
D	0	0	1 (5%)

P03-22 Comparison of metabolic alterations, hepatic and cardiovascular damage between HIV patients with steatosis and primary NAFLD: role of visceral adiposity

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Background and aims: People living with HIV (PLWH) show a high prevalence of hepatic steatosis (HS) and fibrosis, along with frequent metabolic comorbidities with consequent increased cardiovascular (CV) risk. However, if presentation and severity of liver disease, metabolic alterations and cardiovascular complications in HIV patients with HS are different from that of non-alcoholic fatty liver disease (NAFLD) is unknown. The aim is to evaluate prevalence of metabolic alterations, liver and CV damage in PLWH with steatosis, in comparison with those observed in primary NAFLD subjects.

Method: 42 PLWH mono-infected patients (mean age 46±12 ys, male 81%; 90% in viral suppression) were enrolled. PLWH underwent hepatic ultrasound (US) and those with HS were compared to a sex and age matched NAFLD control group (1:2). For all subjects, anthropometric parameters (BMI, waist circumference-WC), metabolic comorbidities, fat mass and sarcopenia by bioimpedance, liver damage by transaminases and Fibroscan (advanced fibrosis≥F3 for LSM>8.9/7.2kPa M/XL probe), CV risk by the European Society of Cardiology (ESC) guidelines 2021, CV damage by carotid ultrasound (plaques, arterial stiffness by radiofrequency as pulse wave velocity-pWV) and heart ultrasound (systolic and diastolic function and epicardial adipose tissue-EAT considered increased for values >9.5/7.5mm in males/females) were assessed. Genotyping for PNPLA3 was determined by Taqman assay.

Results: Thirty (71%) HIV patients presenting HS were compared with 60 NAFLD patients. PLWH with HS presented lower BMI (27.1±4 vs 29.1±4.3 kg/m², p = 0.04), WC (98±9 vs 103.1±10.3 cm, p = 0.03) and trunk fat mass (9.8±3.3 vs 12.4±4.7 kg, p = 0.02) compared to primary NAFLD. Nevertheless, prevalence of metabolic alterations (type 2 diabetes 13% vs 13%, p = 1.0; hypertension 47% vs 42%, p = 0.82; dyslipidemia 83% vs 85%, p = 1.0) and sarcopenia (40% vs 52%, p = 0.81) was not significantly different between groups, as well as of severity of liver damage (increased transaminases 17% vs 20%, p = 0.78; fibrosis ≥F3 17% vs 12%, p = 0.53). Similarly, PLWH with HS and primary NAFLD patients showed the same prevalence of carotid plaques (39% vs 28%, p = 0.33), increased EAT (20% vs 17%, p = 0.77), systolic (6% vs 5%, p = 1.0) and diastolic dysfunction (7% vs 6%, p = 1.0), as well as similar pWV values (7.4±2 vs 6.9±1.4 m/s, p = 0.18). Finally, PNPLA3 distribution was not significantly different between groups (p = 0.16).

Conclusion: Hepatic steatosis and fibrosis are highly prevalent in patient with HIV mono-infection. Interestingly PLWH with HS have similar prevalence of metabolic alterations, liver and CV damage compared to primary NAFLD, despite presenting lower BMI and visceral adiposity. Therefore, screening for steatosis and follow-up of hepatic and CV complications in HIV patients is mandatory independently of body weight or visceral adiposity.

P03-24 Steroidomic profile is associated with adipose tissue insulin resistance and severe liver fibrosis in non-diabetic patients with non-alcoholic fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is characterized by insulin resistance (IR) in the adipose tissue (AT), which is implicated in the progression of liver damage. Steroids may affect lipid metabolism but their pathophysiological role in the setting of NAFLD remain to be fully explored. We aimed to investigate the potential crosstalk between steroidomics, IR and liver damage in a group of non-diabetic subjects with biopsy-proven NAFLD.

Method: We studied 52 patients (median age 44 years [range 20-70], 25% male) who underwent an oral glucose tolerance test with [2H5] glycerol tracer in fasting conditions. AT-IR was calculated as Glycerol rate of appearance (Ra)*insulin (Lipo-IR) and as free fatty acids (FFAs)*insulin (AT-IR). At baseline, we measured a panel of 26 steroids (including glucocorticoids, mineralocorticoids, androgens, progestogens and their representative glucuro- and sulphoconjugated metabolites) by liquid chromatography coupled to mass spectrometry. Severe hepatic fibrosis was defined by F_≥3 (N = 13, 25%).

Results: Glycerol Ra was inversely correlated with testosterone (T) ($r_s = -0.29$, $p = 0.033$) and dihydrotestosterone (DHT) ($r_s = -0.30$, $p = 0.029$). Coherently, T and DHT levels were inversely correlated with FFAs levels ($r_s = -0.29$, $p = 0.035$ and $r_s = -0.34$, $p = 0.013$), with AT-IR ($r_s = -0.31$, $p = 0.023$ and $r_s = -0.29$, $p = 0.038$) and with Lipo-IR ($r_s = -0.31$, $p = 0.023$ and $r_s = -0.30$, $p = 0.033$). Concerning liver histology, T and DHT levels inversely correlated with the amount of hepatic fat ($r_s = -0.37$, $p = 0.008$ and $r_s = -0.41$, $p = 0.003$). Adipo-IR and Lipo-IR increased according to the degree of hepatic fibrosis (ANOVA, $p = 0.002$ and $p = 0.05$, respectively). Conversely, cortisone, 17 α -hydroprogesterone, 21-deoxycortisol, 5 β -androstane-3 α , 17 β -diol 3-glucuronide, T and DHT levels were inversely correlated with the degree of hepatic fibrosis. At multivariable logistic regression analysis adjusted for age, gender, BMI and AT-IR, cortisone levels remained the only steroid significantly associated with F_≥3 (OR = 0.80, 95%CI = 0.69-0.94, $p = 0.006$).

Conclusion: In non-diabetic NAFLD patients, alterations in androgens levels reflect a deranged metabolic milieu. Among glucocorticoids, cortisone levels are associated with severe fibrosis, suggesting an involvement in the progression of liver damage.

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P03-25-YI Late chronotype is associated with significant liver fibrosis in patients with non-alcoholic fatty liver disease

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Background and aims: Late chronotype, i.e. an individual's aptitude to perform daily activities late in the day, has been associated with metabolic syndrome. The aim of this work was to investigate the potential association between chronotype and risk of significant liver fibrosis (F \geq 2) in individuals with non-alcoholic fatty liver disease (NAFLD).

Method: Between 2016 and 2021, 130 patients with a diagnosis of NAFLD by ultrasound were consecutively enrolled. All patients underwent liver stiffness + controlled attenuation parameter (CAP) assessment (FibroScan® 530). F \geq 2 was defined by liver stiffness values \geq 7.1 kPa. The chronotype (MSFsc) was defined by the Munich Chronotype Questionnaire (MCTQ) as the mid-sleep on free days (MSF) corrected for sleep debt on working days, and was expressed as h:min. According to the median value of MSFsc in our cohort, we defined early and late chronotype. In addition, average weekly sleep duration, sleep duration on workdays, sleep duration on free days (SDF) and mid-sleep on workdays (MSW) were recorded.

Results: Median age was 56 (47-63) years and the 56.2% of participants were male. Median body mass index (BMI) was 29.6 (26.9-32.3) kg/m². The principal comorbidities were type-2 diabetes mellitus (T2DM) (n = 37; 28.5%), arterial hypertension (n = 75; 57.7%), dyslipidemia (n = 88; 67.7%), obstructive sleep apnea (OSAS) (n = 9; 6.9%) and depression (n = 10; 7.7%). Median liver stiffness and CAP values were 5.3, 4.4-6.3 kPa (F \geq 2: n = 25; 19.2%) and 304, 265-342 db/m, respectively. The prevalence of late chronotype was significantly higher in patients with F \geq 2 as compared to those without significant liver fibrosis (68.0% vs. 45.7%, p = 0.045; MSFsc [h:min] 4:06 vs. 3:38, p = 0.013). Remarkably, at multivariate analysis adjusted for sex, age, BMI, T2DM, OSAS, arterial hypertension, dyslipidemia, and depression, only T2DM (OR = 11.88, 95%CI 3.25-43.41, p < 0.001) and late chronotype (OR = 4.96, 95%CI 1.38-17.83, p = 0.014) resulted significantly and independently associated to F \geq 2. In addition, patients with F \geq 2 showed lower SDF (h:min) (7:15 vs. 7:50, p = 0.015) and higher MSW (h:min) (3:55 vs. 3:15, p = 0.007).

Conclusion: We observed that late chronotype was associated with significant liver fibrosis in patients with NAFLD, suggesting its potential role in the pathogenesis of this complex disease.

Italian Ministry for Education, University and Research (MIUR) under the programme "Dipartimenti di Eccellenza 2018-2022" Project code D15D18000410001.

P04-01 Biomarkers, imaging, and safety in a well-compensated NASH cirrhotic cohort treated with resmetirom, a thyroid hormone receptor-beta selective agonist, for 52 weeks

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Background and aims: MAESTRO-NAFLD-1 (NCT04197479) is a 52-week randomized double-blind placebo-controlled Phase 3 trial to evaluate the safety and biomarker effects of resmetirom, a liver-targeted, oral, once-daily thyroid hormone receptor-beta selective agonist, in >1200 NASH patients with F1-F4 fibrosis (identified using non-invasive biomarkers and imaging). A goal of this “real-world” NASH study is to identify non-invasive markers that correlate with individual patient response to resmetirom treatment. The study includes an open-label resmetirom arm in well-compensated NASH cirrhotic patients.

Method: Eligibility required ≥ 3 metabolic risk factors (metabolic syndrome) and NASH cirrhosis (diagnosed on liver biopsy or according to accepted criteria). The primary and key secondary end points include safety, relative percent reduction in MRI-PDFF (Week 16), LDL-C (Week 24), apoB, and triglycerides, and markers of fibrosis. Patients received 80-100 mg resmetirom daily for 52 weeks.

Results: 105 well-compensated NASH cirrhotic patients were enrolled in the open-label arm; 2/3 confirmed by liver biopsy. 60% had completed Week 52 at the time of this abstract. Demographic and baseline characteristics include mean age 62.7 years (SD, 9.0), 64% female, BMI 35.4 kg/m² (7.4), 70% diabetes, 77% hypertension, >70% dyslipidemia, mean ASCVD score 16.1%, 32.4% hypothyroid, 51% on statins. MRE 5.7 kPa (2.1); FibroScan 24.6 kPa (14.9)/CAP 318 (59); and mean MRI-PDFF 8.1% (5). Stage of cirrhosis was inversely correlated with baseline PDFF. At Week 52, resmetirom lowered FibroScan CAP (by 42 units [$p < 0.0001$]) and kPa (by 7.6 kPa [$p = 0.02$]). In patients with baseline PDFF >5% (5% = UL normal), resmetirom lowered PDFF by 37% ($p < 0.0057$). Resmetirom lowered MRE by 0.68 kPa at Week 52; 34% of patients had an MRE reduction $\geq 15\%$. GGT and ALP were reduced (by 27% and 18%, respectively; $p = 0.04$ for both). Liver volume, which was elevated at baseline, was reduced 15.9% (7.7%) at Week 16 ($p < 0.0001$) independent of baseline PDFF. Liver volume reduction correlated with reduction in MRE, MRI-PDFF, TIMP, P3NP, and SHBG (**Table**). Resmetirom reduced LDL-C (20%), apoB (20%), triglycerides (21%), and Lp (a) (30%) independent of cirrhosis stage. BP was reduced by 4-5mmHg. Resmetirom was well tolerated.

Conclusion: Resmetirom treatment for up to 52 weeks was effective at lowering markers of cardiovascular risk and NASH fibrosis in patients with well-compensated NASH cirrhosis.

Figure:

Table.				
	Pearson		Spearman	
CFB in:	%CFB LV	CFB LV	CFB LV	P value
TIMP	0.500	0.350	0.369	0.007
P3NP	0.351	0.294	0.238	0.086
MRI-PDFF (Week 16)	0.331	0.438	0.371	0.006
MRE (Week 16)	0.327	0.273	0.347	0.014
AST (Week 52)	0.197	0.254	0.191	0.170
SHBG	-0.282	-0.274	-0.344	0.012
AST, aspartate aminotransferase; CFB, change from baseline; LV, liver volume; MRE, magnetic resonance elastography; MRI-PDFF, magnetic resonance imaging-proton density fat fraction; TIMP, tissue inhibitor metalloproteinase; P3NP, amino terminal procollagen peptide; SHBG, sex hormone binding globulin.				

P04-02 Multi-omic machine learning panel accurately stage fibrosis in asian patients with NAFLD: a prospective derivation and validation study

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease worldwide. Liver fibrosis is strongly associated with adverse clinical outcomes in people with NAFLD; however, early detection of liver fibrosis remains a challenge.

Method: We conducted a multi-omics study, integrating the proteome, metabolome, and lipidome in every serum, urine, and stool sample from 166 patients with liver biopsy-proven NAFLD and 62 non-steatotic healthy volunteers. We trained and tested diagnostic models by machine learning methodology in the derivation cohort and then validated them in an independent validation cohort.

Results: By a least absolute shrinkage and selector operation algorithm, we established three serum multi-omic diagnostic panels that showed excellent performances in detecting NAFLD, any stage of fibrosis, and significant fibrosis with receiver operator characteristic areas under the curves of 1.0, 0.943, and 0.988 in the training cohort, respectively. Similar results were found in the testing and independent validation cohorts. However, biomarkers identified in urine and stool samples did not show good performance for diagnosing NAFLD or fibrosis.

Conclusion: Our study provides a comprehensive understanding of the molecular changes in NAFLD and our multi-omic machine learning models could be used for the non-invasive diagnosis of liver fibrosis.

Figure:

Figure 1. Definition of high-confidence serum biomarkers for the diagnosis of NAFLD in both the training cohort and testing cohort. Figure 2. Definition of high-confidence serum biomarkers for the diagnosis of NAFLD with presence of liver fibrosis in both the training cohort and testing cohort.

P04-03-YI Effects of coping, quality of life and liver fibrosis on self-efficacy of patients with non-alcoholic fatty liver disease

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Background and aims: Patients with non-alcoholic fatty liver disease (NAFLD) have been found to have lower self-efficacy, i.e. less confidence in managing their disease and making changes in health-related behaviour than other chronic liver disease patients. However, the influence of psychological variables such as coping or quality of life on self-efficacy, or the role of liver fibrosis in these relationships in NAFLD patients have not yet been studied. Therefore, our objectives were to: 1) Find out whether mental and physical quality of life mediate the relationship between passive/avoidance coping and self-efficacy, and 2) Test whether liver fibrosis exerts a moderating effect on this relationship.

Method: We assessed 509 biopsy-proven NAFLD patients (300 men and 209 women, mean age 55.07 ± 11.85) using the 12-Item Short-Form Health Survey (SF-12), Brief COPE (COPE-28) and General Self-Efficacy Scale (GSE). The passive/avoidance coping variable was constructed using patients' mean scores on the denial, self-blame, disengagement and self-distraction coping strategies. For the first objective, Model 6 mediation was performed using the SPSS PROCESS v3.5 macro, while for the second objective, Model 91 was used for moderated mediation. In both models, 5000 bootstrap samples were employed to test the indirect effects estimated, which were considered significant when the confidence interval (CI) at 95% did not include 0.

Results: Mental and physical quality of life mediated the association between passive/avoidance coping and self-efficacy (effect = -1.435, CI = -2.356 to -0.661). The direct effect of passive/avoidance coping on self-efficacy was significant after mediation analysis (effect = -9.625, $p < 0.001$), showing partial mediation of mental and physical quality of life. Liver fibrosis (beta = 0.367, $p < 0.001$) moderated the relationship between mental and physical quality of life. The indirect conditional effects of passive/avoidance coping on self-efficacy through mental and physical quality of life were higher in patients who had significant fibrosis than those who did not.

Conclusion: These results showed that coping strategies and quality of life are two psychological biomarkers influencing the self-efficacy of NAFLD patients. In addition, the presence of significant fibrosis was found to be a risk factor associated with lower self-efficacy. In conclusion, high passive/avoidance coping and impaired quality of life could partly explain the low self-efficacy of NAFLD patients usually observed. Considering the negative impact of low self-efficacy on therapeutic adherence in chronic diseases, these results emphasize the importance of incorporating emotional and cognitive aspects in NAFLD evaluation and treatment, especially for patients with significant fibrosis.

P04-09-YI Lipid profiling of extracellular vesicles and plasma in people with non-alcoholic fatty liver disease

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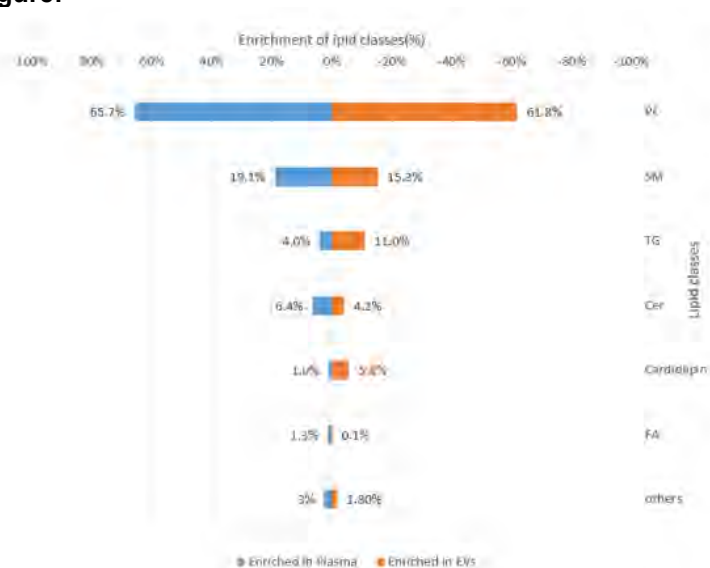
Background and aims: Non-alcoholic fatty liver disease (NAFLD), as an aspect of metabolic syndrome, is one of the most common liver diseases worldwide. Lipid metabolism is related to the progression of NAFLD to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis. Extracellular vesicles (EVs) carrying a range of molecules, including lipids, could play a role in this progression via modulating injury, amplifying inflammation and promoting liver fibrosis. We aimed to characterise the lipid profile in plasma and plasma-EVs in NAFLD patients.

Method: Plasma samples were obtained from 60 NAFLD patients and 20 healthy controls. EVs were isolated via the Total Exosome Isolation Reagent (Thermo) from plasma and were identified via transmission electron microscopy. The lipids extracted from plasma and EVs were analysed via ultra-high performance liquid chromatography/ion mobility time-of-flight mass spectrometry (UHPLC/IM-QTOF-MS)-based untargeted lipidomics. Data was processed via a KniMet pipeline and R. Statistics analysis was performed via SIMCA and GraphPad Prism.

Results: 25 females and 35 males with a histological diagnosis of NAFLD were included, with 30 NAFLD patients aged more than 55 years. 21 NAFLD patients had fibrosis. 545 lipids in plasma and 610 lipids in EVs were detected and identified via LIPIDMAPS accurate mass search and MSMS fragmentation. Plasma contained phosphatidylcholines (PC), sphingomyelins (SM), ceramides (Cer), and triacylglycerols (TG) in both NAFLD and healthy groups, while EVs contained more TG and cardiolipin than whole plasma samples. In both plasma and EVs, SM d34:1, SM d38:1, SM d40:1, SM d40:2, SM d41:2 and SM d42:3 were decreased, and PC (40:6) was more abundant in NAFLD patients compared with healthy individuals. In NAFLD patients, PC (36:2) and PC (36:4) were increased in EVs, while decreased in plasma. SM d42:2 was decreased in EVs and increased in plasma.

Conclusion: Our study revealed a difference in lipidomic profile between plasma and EVs in patients with NAFLD. PC (36:2) and PC (36:4) were enriched in EVs but decreased in plasma. The opposite trend of PC (36:2) and PC (36:4) could be related to the pathogenesis of disease progression.

Figure:



In EVs	In Plasma
SM d34:1↓	SM d18:1_16:0↓
SM d34:2↓	SM d34:2
SM d16:1_22:0↓	SM d38:1↓
SM d40:1↓	SM d40:1↓
SM d40:2↓	SM d40:2 ↓
SM d41:2↓	SM d41:2↓
SM d42:2↓	SM d42:2 ↑
SM d42:3↓	SM d42:3↓
PC 16:0_18:2↓	PC 16:0_18:2
X	PC 16:0_18:3 ↓
X	PC 36:5 ↑
X	PC 16:0_20:3 ↑
PC 18:0_18:2↑	PC 18:0_18:2 ↓
PC 18:2_18:2↑	PC 18:2_18:2↓
PC 18:0_22:6↑	PC 18:0_22:6↑
X	LPC 16:0/0:0 ↑
X	LPC 0:0/18:1 ↑
X	EtherPC 16:1e_18:2↓

↑ more abundant ↓ less abundant in NAFLD group compared with Healthy group. X no detected in group.

P04-10 Non-invasive tests for non-alcoholic fatty liver disease (NAFLD) in a multi-ethnic population. The Helius study

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is increasing in prevalence and severity globally, prompting non-invasive testing, yet limited data exist on non-invasive liver tests (NITs) including vibration controlled transient elastography (VCTE) in ethnically diverse populations. Therefore, we studied prevalence and ethnic differences in NAFLD with NITs in the multi-ethnic HELIUS cohort in The Netherlands.

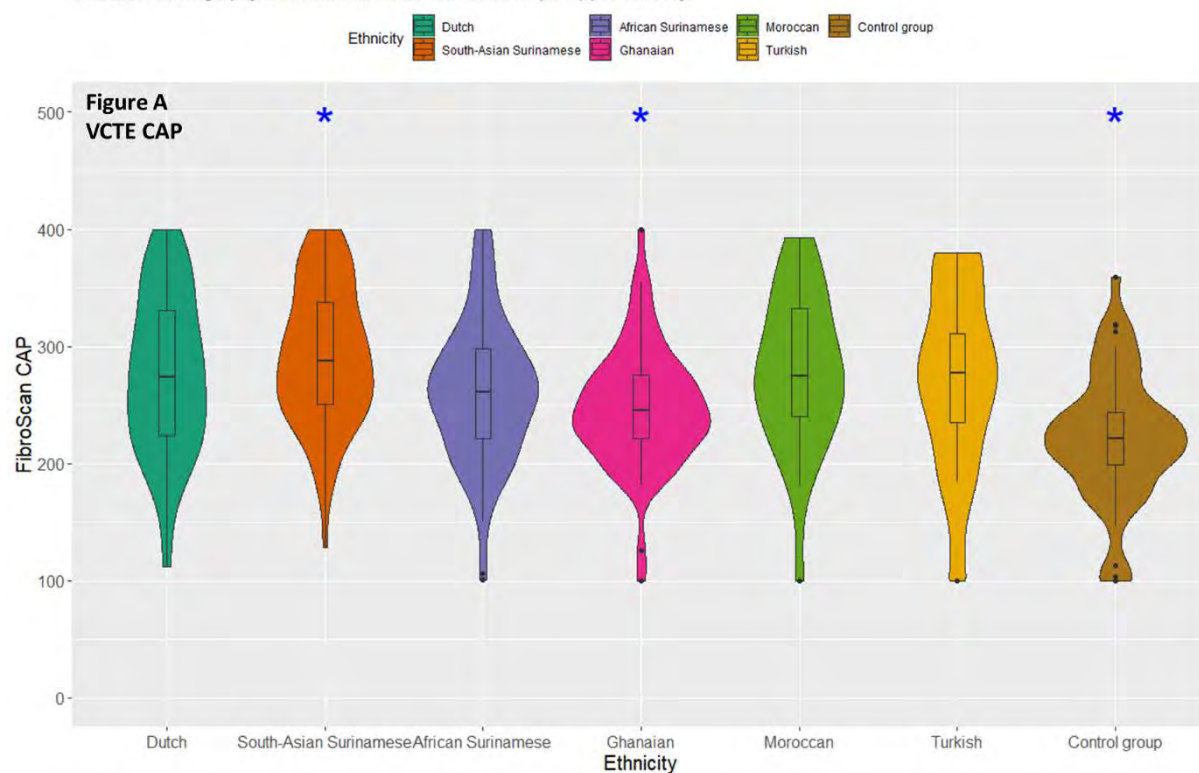
Method: NITs of liver steatosis-Fatty Liver Index (FLI)-and fibrosis-Fibrosis 4 index (FIB4) and AST to platelet ratio (APRI)-were assessed in 10,007 HELIUS participants. A subpopulation of 399 participants, selected on high-risk criteria for NAFLD (obesity, T2DM and/or elevated NITs), was examined with VCTE.

Results: FLI was ≥ 60 in 27.3% of 10,007 participants, indicating steatosis. Most participants (71.8%) had FIB4 < 1.30 , excluding advanced liver fibrosis, and 1.1% ($n = 113$) had high FIB4 (FIB4 ≥ 2.67), indicating likely advanced liver fibrosis. In the VCTE subpopulation, 37.8% and 17.3% had steatosis and fibrosis (continuation attenuation parameter (CAP) ≥ 280 dB/m, liver stiffness measurement (LSM) ≥ 7.0 kPa, respectively). Turkish participants had highest adjusted OR for elevated LSM (1.72, 95%CI 0.59-5.01) and Ghanaians lowest (0.24, 95%CI 0.09-0.65). Ghanaians had lowest adjusted OR for elevated CAP: 0.18 (95%CI 0.09-0.37). In diabetics, CAP and LSM were higher than in non-diabetics (300dB/m versus 255dB/m and 5.50kPa versus 4.80kPa, respectively, $p < 0.01$).

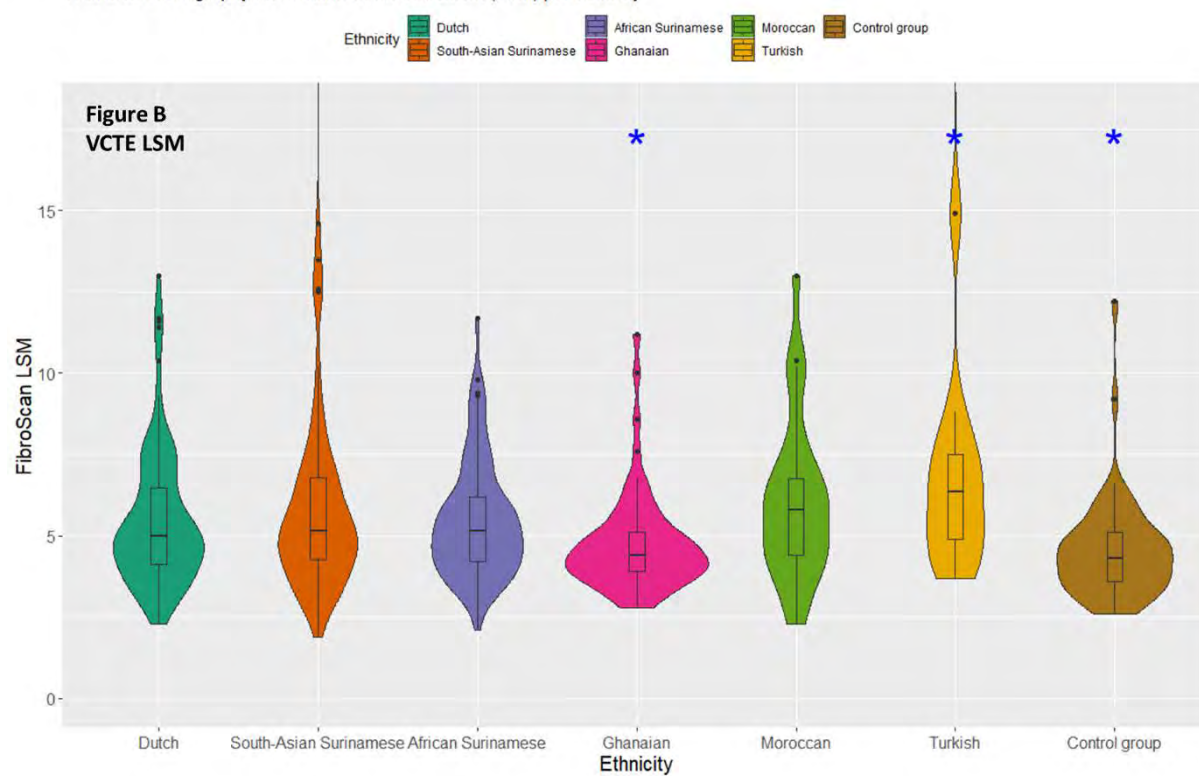
Conclusion: Liver steatosis proxy FLI was elevated in 27.3% of this multi-ethnic population. In Turkish background and in those with T2DM, proxies for steatosis and fibrosis were high, whereas in Ghanaian background, NITs were generally low. Together, this warrants clinical awareness for NAFLD among high-risk populations, taking ethnic background into account.

Figure: Violin plot of **A**) VCTE CAP **B**) VCTE LSM in the VCTE substudy participants stratified per ethnicity, including the control group. Significance was calculated per ethnicity in comparison with the metabolic risk group ($n = 346$). In addition the median LSM in the control group were both significant different from the metabolic risk group

Transient Elastography Controlled Attenuation Parameter (CAP) per ethnicity



Transient Elastography Liver Stiffness Measurement (LSM) per ethnicity



*indicates significant different compared to metabolic risk group (n = 346)

P04-11 Improving diagnostic performance of liver fibrosis, NASH and NAFLD activity score (NAS) with 3D vector magnetic resonance elastography (MRE)

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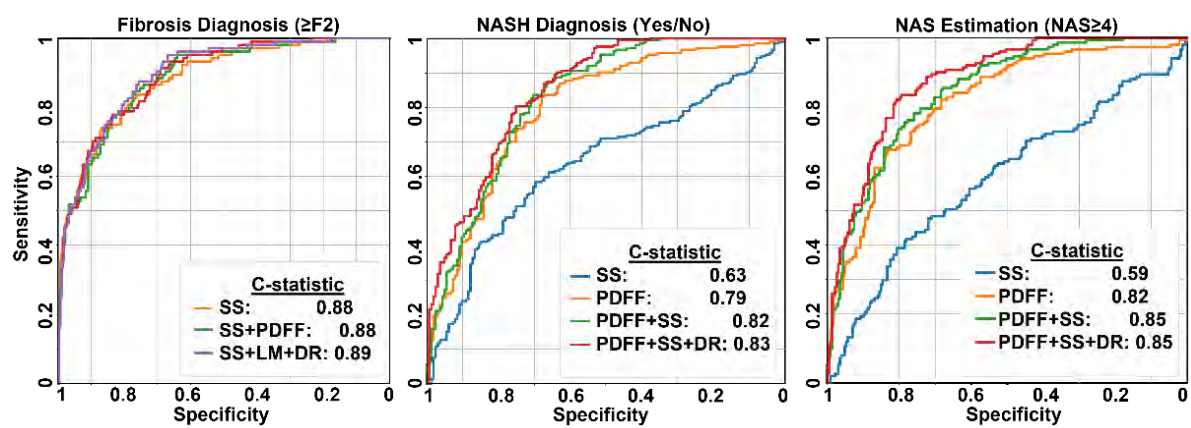
Background and aims: Liver stiffness or shear stiffness of the liver has been widely used for quantifying hepatic fibrosis in patients with chronic liver diseases. Besides liver stiffness, 3D vector MRE can offer many other mechanical parameters, including viscosity-associated loss modulus (LM) and damping ratio (DR) to comprehensively characterize hepatic abnormalities such as inflammation. In clinical management of non-alcoholic fatty liver disease (NAFLD), fat characterization with proton-density fat fraction (PDFF) technique of magnetic resonance imaging (MRI) and MRE are promising techniques for diagnosing and monitoring NAFLD development and progression. This study aimed to develop models using single frequency 3D vector MRE at 60Hz and MRI to improve fibrosis staging, NASH diagnosis, and NAFLD activity score (NAS) prediction.

Method: We pooled three prospective clinical studies across two medical centers (site 1: 277, site 2:102) in 379 biopsy-proven NAFLD patients who had 3D vector MRE and MRI performed within 90 days of liver biopsy. Imaging parameters were extracted from two series that comprised of 6-point Dixon MRI (one breath-holding) and 3D vector MRE at 60Hz (three breath-holding). In 3D MRE exams, besides shear stiffness (SS), loss modulus (LM) and damping ratio (DR) were also calculated. Histopathological readings of fibrosis stage (F0-4), NASH diagnosis (Yes/No) and NAS score (0-8) were targeted as three different ordinal/categorical outcomes for our multivariate logistic regression models. We applied leave-one-out iterative cross-validation for all models with 4 imaging predictors and their different combinations. C-statistic was reported for each model. We used DeLong test to evaluate the significance of model improvement in diagnostic accuracy.

Results: The figure below shows the improved performance of the single- and multi-parametric predictive models. Of the models trained, the three-parameter models performed well for all responses (C-statistic = 0.895, 0.849, and 0.853 for significant fibrosis, NASH, and NAS ≥ 4 respectively). For diagnosing significant fibrosis, single parameter model SS provided sufficiently high accuracy at 0.88. When differentiating NASH, the three-parameter model PDFF+SS+DR showed superior performance (C-statistic = 0.84) than two-parameter model PDFF+SS (C-statistic = 0.82, $p < 0.001$) or single-parameter model alone (C-statistic = 0.79, $p < 0.001$). A similar trend occurs between three-, two- and single-parameter models in NAS prediction.

Conclusion: Single parameter of MRE-assessed liver stiffness model is very accurate in diagnosing fibrosis in NAFLD. Additionally, multiple parameters of MRI/MRE-assessed PDFF, SS and LM or DR model can significantly improve diagnostic accuracy in NAFLD management, especially in NASH diagnosis and NAS prediction.

Figure:



P04-12 Pegzofermin improved liver histology, liver-related non-invasive tests (NITS) and metabolic profiles in an open-label cohort of a phase 1b/2a study in subjects with non-alcoholic steatohepatitis

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Background and aims: Pegzofermin (PGZ), a long-acting glycoPEGylated recombinant human FGF21 analog, had significant liver-related and cardiometabolic benefits, with favorable safety and tolerability, in a Phase 1b/2a study in NASH.

Method: In this open label cohort, 20 subjects with biopsy-proven NASH (NAS ≥ 4 , fibrosis stage F2/F3) received SC PGZ 27 mg QW for 20 weeks. Key end points were histology [≥ 2 -point improvement in NAS with ≥ 1 point improvement in ballooning or inflammation; NASH resolution without worsening of fibrosis, and ≥ 1 stage fibrosis improvement without worsening of NASH], and safety and tolerability at week 20 (W20). Change in liver fat and liver and metabolic markers were also assessed. Biopsies were read by one central reader.

Results: BL characteristics included (means): Age 58.4 years, BMI 37.0 kg/m², NAS 5.3 points, MRI-PDFF 21.1%, ALT 47.1 U/L, ProC3 19.3 ng/ml and Fibroscan™ VCTE score 14.3 kPa. 75% of subjects were female, 85% had T2DM, and fibrosis stage was F3 in 65% and F2 in 35% of subjects. 19/20 subjects completed treatment and had W20 biopsies. At W20, 74% of subjects had ≥ 2 pt reduction in NAS (mean absolute change -2.4 pts); 32% and 26% had NASH resolution without fibrosis worsening and improved fibrosis without NASH worsening, respectively. There was a consistent improvement in NITs that assess fibrosis compared to BL [VCTE score -4.6 kPa (-31%; $p < 0.001$), FAST score -0.47 (-76%; $p < 0.001$), FIB4 -0.29 (-19%; $p < 0.01$) and ProC3 -4.3 ng/ml (-20%; $p < 0.001$). 88% of subjects with BL FAST scores in the 'rule-in' or 'indeterminate' range had 'rule-out' FAST scores (≤ 0.35) at W20. At W20, mean relative reduction in MRI-PDFF was 65% ($p < 0.001$), and 100% and 79% of subjects had $\geq 30\%$ and $\geq 50\%$ reductions, respectively. ALT decreased by 46% compared to BL ($p < 0.001$); mean absolute decrease in ALT was -27.8 U/L in subjects with elevated ALT at BL, and ALT decreased ≥ 17 U/L in 71% of these subjects. At W20, significant improvement in HbA1c and lipids, weight loss and increased adiponectin were observed in PGZ-treated subjects. PGZ was safe and well tolerated. There were no deaths, related SAEs or discontinuations due to AEs. Treatment related AEs reported in $\geq 10\%$ of subjects included diarrhea, nausea, vomiting, injection site bruising, injection site erythema and decreased appetite; there were no reports of AEs grade ≥ 3 , hypersensitivity, tremor or adverse effects on vital signs.

Conclusion: PGZ (27 mg QW for 20 weeks) led to meaningful changes in key histology end points and fibrosis-related NITs, reduction in MRI-PDFF and decreased ALT in a cohort of NASH subjects with advanced fibrosis. In addition to these benefits on liver health, PGZ significantly improved metabolic parameters, with good safety and tolerability. These results extend the growing evidence of PGZ's potential as treatment for NASH. PGZ is currently being evaluated in NASH in the ongoing Phase 2b ENLIVEN study.

P04-13 NAFLD is associated with significantly higher risk of cardiovascular outcomes in the absence of fibrosis-a prospective UK Biobank analysis in 33, 616 individuals

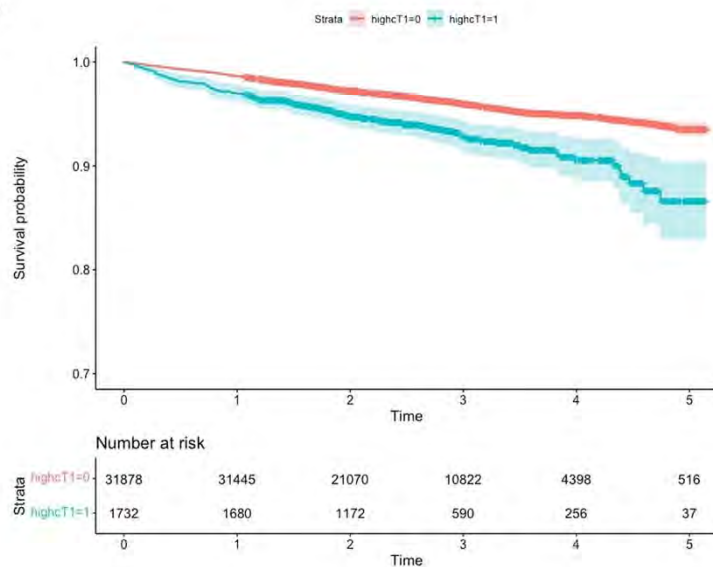
Adriana Roca-Fernandez¹, Anneli Andersson¹ Matt Kelly¹, Celeste McCracken², Helena Thomaides-Brears¹, Andrea Dennis¹, Betty Raman², Rowan Nicholls¹, Stefan Neubauer², Steffen Petersen³, Zahra Raisi-Estabragh³, Thomas Nichols⁴, Ntobeko Ntusi⁵, Kazem Rahimi⁶, Rajarshi Banerjee¹, Amitava Banerjee⁷

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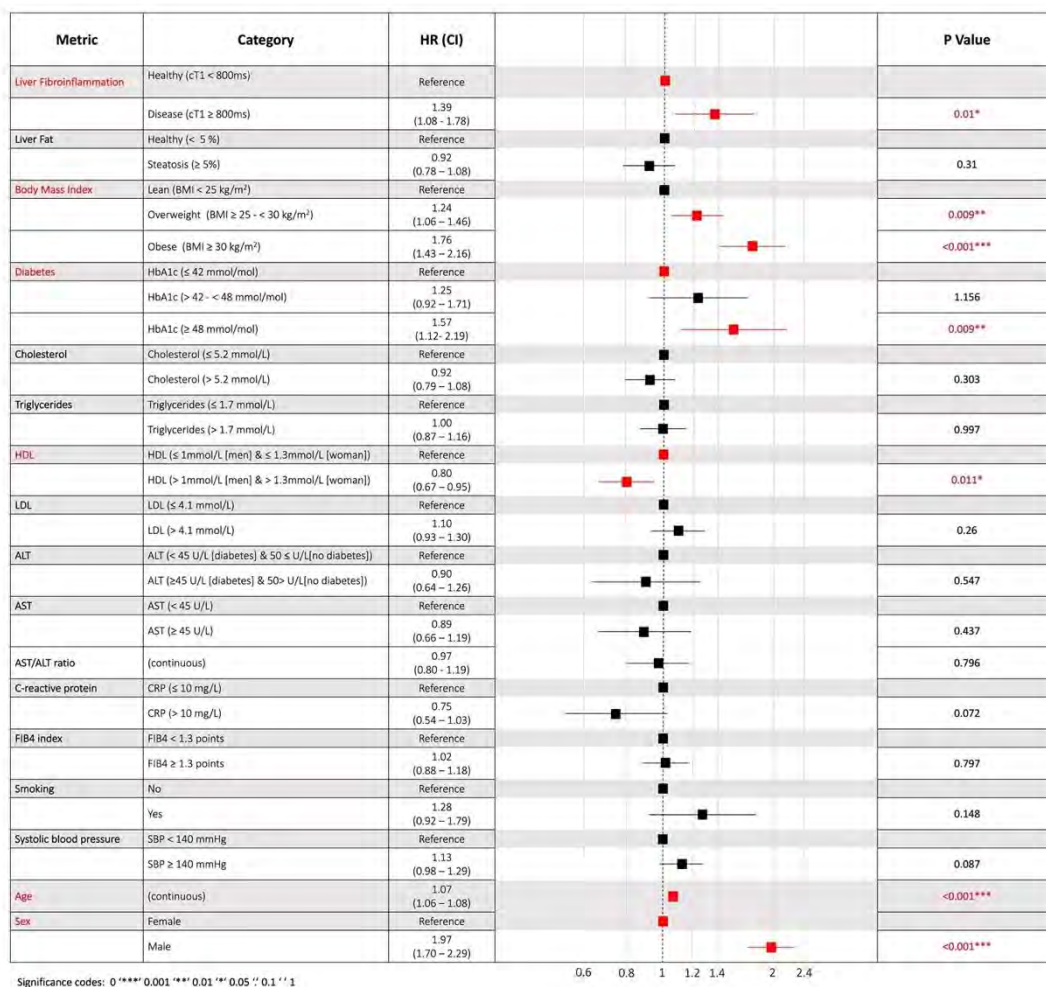
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Abstract under embargo until 15 September 2022.

A)



B)



P04-15 MRI assessment (cT1) with LiverMultiScan following VCTE improves the diagnostic yield for high-risk NASH

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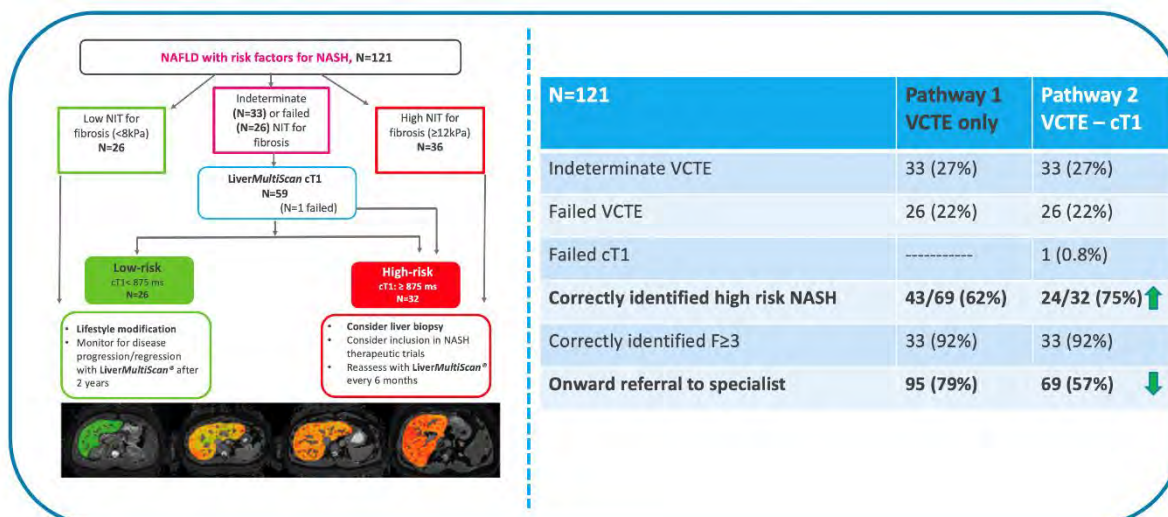
Background and aims: In the diagnostic pathway for NAFLD, EASL recommends the use of non-invasive tests (NITs) for assessing fibrosis. However, NASH, independently of fibrosis stage, is also associated with poor outcomes and thus high costs of care. Accordingly, recent EASL and NICE guidance recommend diagnosis and management of this patient group. As an MRI-derived measure, cT1 is an excellent biomarker to diagnose NASH, shown to predict clinical outcomes, and has become a leading NIT for stratifying patients based on high-disease risk. We compared a clinical pathway using cT1 for stratifying high-risk NASH to the current recommendation using NITs for advanced fibrosis only, to explore diagnostic accuracy and appropriate further referral to specialist care.

Method: We studied NAFLD patients who had undergone NITs; liver stiffness (kPa) from vibration controlled transient elastography (VCTE, FibroScan®), and MRI-derived cT1 (ms) with LiverMultiScan®. All had liver fat ≥5% (LiverMultiScan® PDFF) and had histologically confirmed NAFLD with liver biopsy. We compared two diagnostic approaches: (1) Utilising VCTE results only, to exclude low risk individuals and identify those with advanced fibrosis suitable for onward referral; (2) VCTE followed by cT1 (for NASH risk stratification). We assessed the number of failed and indeterminate results, % positive predictor value (PPV) for high-risk NASH cases correctly identified, and number referred onto specialist care. Correct identification of fibrosis was based on F≥3; “high-risk NASH” was NAS≥4 and F≥2; established (EASL guidelines) VCTE thresholds were <8 kPa rule out and ≥12 kPa rule in; cT1 threshold was ≥875ms.

Results: Using data from N = 121 patients (60 yrs., 40% female, 59% BMI ≥27 kg/m²). The second pathway (VCTE followed by cT1) increased the PPV for high-risk NASH from 62% (43/69 with VCTE≥8kPa) to 75% (24/32 with cT1 ≥875ms) and reduced subsequent referrals from 95 to 69 individuals. This was driven by better stratification of failed or indeterminate cases, and greater specificity for the identification of those with high-risk NASH by incorporating cT1 (Figure 1).

Conclusion: Investigating suspected advanced fibrosis using positive NIT results, with combined TE and MRI assessment of cT1 increased the diagnostic accuracy of high-risk NASH by 21% and reduced liver biopsy by 28%. Screening tests based on NITs for fibrosis alone are inadequate for risk stratification and diagnosis of NASH. Inclusion of MRI-assessed cT1 in NAFLD/NASH clinical diagnostic pathways improves diagnostic accuracy and reduces the need for biopsy and for specialist referral, which would result in downstream cost savings.

Figure:



P04-16-YI Prevalence of subclinical cardiovascular disease in patients with non-alcoholic fatty liver disease. Analysis of the Paracelsus 10.000 cohort study

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Background and aims: Recent studies have shown that non-alcoholic fatty liver disease (NAFLD) has a strong correlation with cardiovascular (CV) disease, events, and high mortality from CV causes. In NAFLD patients, cardiovascular disease (CVM) is more often the cause of death than the liver disease itself. However, epidemiological data on the prevalence of atherosclerotic manifestations in NAFLD on a general population background is still inconclusive. Therefore, the aim of this study was to perform a cross-section analysis to show the relationship between NAFLD and coronary artery calcium (CAC) scoring as a surrogate marker for cardiovascular disease in a large population.

Method: This study included 1.760 patients from the Paracelsus 10.000 study. NAFLD was defined by the FLI score. Liver fibrosis was non-invasively estimated using the FIB-4 score. The CAC score was used as a surrogate marker for CVD and was calculated using the Agatston scoring method. Subjects were stratified into a group with CAC (CAC score >0) and without (CAC score = 0). Further, SCORE 2 was calculated according to European Society of Cardiology guidelines to estimate the 10-year risk of cardiovascular disease. To assess the association between NAFLD and CAC, multivariable logistic regression modules were performed. The outcomes were adjusted by three different models: Model 1 describes the univariate logistic regression, Model 2 was adjusted for SCORE 2 alone and Model 3 for age, sex and metabolic syndrome (MS).

Results: In the univariate analysis (Model1) the FLI score was associated with a higher risk for CAC (Results are shown in Table 1.). The same applied for the FIB-4 score in Model 1. In Model 2, the FLI- and FIB-4- Score remained associated with CAC. However, in Model 3, no independent relationship between FLI score and CAC or between the FIB- 4 score and CAC was found.

Conclusion: This study demonstrated that patients with an increasing FLI- and FIB-4- Score as a surrogate for NAFLD had a higher overall risk for CAC. This association was also seen after adjustment for traditional cardiovascular risk factors by the SCORE 2. Nevertheless, after adjustment for NAFLD risk factors like age, sex and MS, no independent associations remained. Results indicate that in addition to traditional cardiovascular risk factors such as hypertension, dyslipidaemia or smoking, liver fat accumulation may represent a further independent risk for CAC development. Our data suggest that liver fat assessment may serve as an additional piece of information in CV risk assessment in addition to risk assessed by Score2 variables, while causality remains to be determined.

Figure:

Table 1.	FLI score	FIB-4 score
	OR (95%CI, p value)	OR (95%CI, p value)
Model 1	1.02 (1.02-1.02, p < 0.01)	1.88 (1.39-2.55, p < 0.01)
Model 2	1.01 (1.00-1.01, p < 0.01)	1.78 (1.26-2.51, p < 0.01)
Model 3	1.01 (1.00-1.01, p = 3.64)	1.21 (0.86-1.70, p = 0.28)

P04-17-YI Effect of smartphone-assisted lifestyle modification intervention in MAFLD patients: a randomized controlled trial

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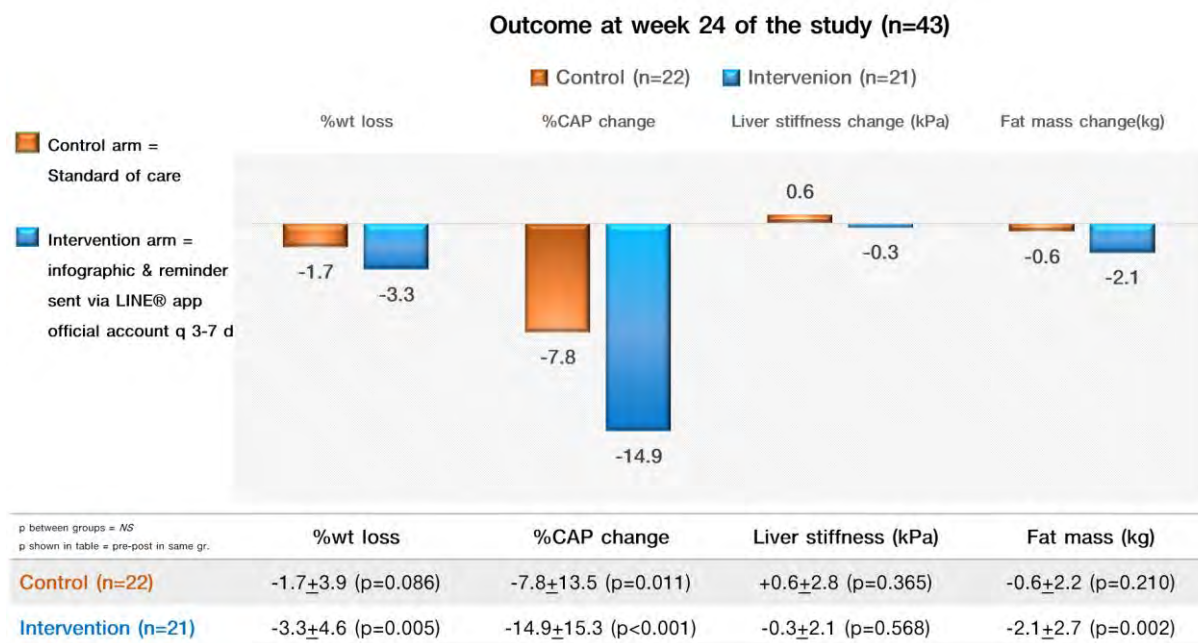
Background and aims: Patients with metabolic-associated fatty liver disease (MAFLD) are on the rise globally. While therapeutic medications are being studied, the mainstay of treatment is lifestyle intervention (LSI), but adherence to LSI and weight loss are difficult to achieve. We hypothesized that providing LSI and MAFLD information, as well as encouraging LSI through a social media application, would improve clinical outcomes in MAFLD more than standard of care (SOC).

Method: This is a preliminary result of a randomized controlled study in noncirrhotic MAFLD patients aged 18-65 years in Thailand. Patients with active malignancy, current pioglitazone treatment, coexisting other liver diseases, and unstable cardiovascular/neurological conditions were excluded. Eligible patients were randomly assigned to control (SOC) and intervention arm. In the control arm, patients receive standard LSI advice from a single hepatologist; whereas in the intervention arm, in addition to SOC, the investigator sent infographics about MAFLD and LSI information and reminded patients to do LSI every 3-7 days via a social media app popular in Thailand (LINE®) using the study's official account. The outcomes are changes in liver steatosis (measured by controlled attenuated parameter) and fibrosis (measured by liver stiffness) by Fibroscan® at 24 weeks, as well as weight loss, body composition, and serum alanine aminotransferase (ALT) level between the two groups.

Results: A total of 122 patients were enrolled. The median age of eligible participants was 53 (IQR: 44.7, 58.8) years, 64.7% were female, median body mass index (BMI) was 27.3 (24.9, 29.9) kg/m², and mean waist circumference was 93.1 ± 9.4 cm. Of all 122 patients enrolled, 43 had been completely followed-up at 24 weeks, 22 and 21 participants in the SOC and intervention group, respectively. The patients in the intervention group experienced a significant reduction in weight, CAP, fat mass, and a significant increase in muscle mass compared to baseline. While the patients in control group also had a reduction in CAP significantly, but not other parameters. The changes in the aforementioned variables between the two groups are shown in the Figure.

Conclusion: In this preliminary result, encouraging LSI and delivering MAFLD information via social media application to patients with MAFLD resulted in a significant reduction in weight, fat mass, and CAP compared to baseline, and the results appeared to be better than SOC. However, the complete data of all eligible patients as well as change in liver steatosis and fibrosis is yet to be confirmed

Figure:



P04-19 Long term safety and efficacy of saroglitazar in NAFLD/NASH population: a prospective, single arm, real world study

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Background and aims: Saroglitazar 4 mg is the only approved drug for use in NAFLD associated with co morbidities and Non Cirrhotic NASH, by DCGI in India. Also, no pharmacotherapy for NASH has been approved so far, by the Food and Drug Administration or European Medicines Agency. The FAST score stratify the disease severity in patients with Non-alcoholic fatty liver disease. This study was aimed to evaluate the long term safety and efficacy of Saroglitazar 4mg in NAFLD and NASH population, in real world scenario.

Method: A total of 54 NAFLD patients diagnosed based on AASLD criteria of NAFLD diagnosis, treated with Saroglitazar 4mg daily for long term (≥ 96 Weeks), were included in the study. The primary end point was to measure the improvement in Fibroscan LSM and CAP parameters. We additionally used the FAST score, a novel index of steatohepatitis, to evaluate the effect of Saroglitazar on disease progression. The statistical significance was established using the paired sample t-test.

Results: The median age was 53.0 (IQR 21.0-78.0) years and 35 patients (65 %) were male. Median body mass index was 26.8 (IQR 19.5-37.5) and waist circumference was 1.01 mtr (IQR 0.81--1.28). Diabetes mellitus and Hypertension were found in 30 (55.5%) and 28 (51.9%) patients, respectively. The Fibroscan LSM value, CAP value and the FAST score improved significantly ($p < 0.001$) and consistently at 24, 48 and 96 weeks of Saroglitazar 4 mg treatment. The measured secondary parameters like AST, ALT, fasting triglyceride and LDL cholesterol also shown highly significant ($p < 0.001$) and consistent improvement during the treatment. There was no therapy related side effects and no discontinuation was observed or reported during the entire treatment duration.

Conclusion: Saroglitazar 4mg treatment is shown to be efficacious and safe in long term treatment. The consistent improvement in the FAST score, shows that Saroglitazar also helpful in arresting the disease progression in NAFLD patients. More controlled and larger clinical studies will throw more light on the evidences.

Figure:

Parameters	Baseline	24 Week	48 Week	96 Week	% Improvement	P Value
LSM (KPa)	14.4 \pm 10.3	11.1 \pm 6.9	9.9 \pm 5.5	8.5 \pm 4.5	41.2 %	p <0.001
CAP (dB/m)	313.1 \pm 41.3	286.8 \pm 44.5	283.4 \pm 43.1	253.9 \pm 52.1	18.9 %	p <0.001
FAST Score	0.58 \pm 0.2	0.4 \pm 0.2	0.31 \pm 0.2	0.26 \pm 0.2	54.2 %	p <0.001
ALT (IU/L)	57.8 \pm 31.5	37.2 \pm 17.9	29.1 \pm 13.3	29 \pm 18.2	49.8 %	p <0.001
AST (IU/L)	52.03 \pm 29.3	38.02 \pm 14	30.6 \pm 11.02	28.9 \pm 10.3	44.3 %	p <0.001
Fasting TG	160.4 \pm 53.9	115.9 \pm 31.1	98.5 \pm 23.3	85.6 \pm 19.1	46.6 %	p <0.001
LDL (mg/dl)	113.1 \pm 26.4	95.8 \pm 27.8	84.1 \pm 18.8	72.4 \pm 20.8	35.9 %	p <0.001

P04-22 Administrative coding for non-alcoholic fatty liver disease is accurate in swedish patients

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Background and aims: Epidemiological studies of non-alcoholic fatty liver disease (NAFLD) frequently use the International Classification of Disease (ICD) coding system to identify patients. However, the validity of using ICD codes for this purpose in a Swedish setting is unknown, and poor accuracy could result in misclassification bias. Here, we aimed to validate the administrative code for NAFLD in Sweden.

Method: In total, 150 patients with an ICD-10 code for NAFLD (K76.0) that had been in contact with the Karolinska University Hospital between 2015-01-01 and 2021-11-03 were randomly selected for inclusion. Patients were classified as true or false positives for NAFLD by medical chart review and the positive predictive value (PPV) for the ICD-10 code corresponding to NAFLD was calculated.

Results: The PPV for the ICD-10 code for NAFLD was 0.82 (95% confidence interval [CI] 0.76-0.89). After exclusion of patients with coding for competing liver diseases or alcohol abuse disorder (n = 14), the PPV was improved to 0.91 (95% CI 0.87-0.96). The PPV was also higher in patients with coding for NAFLD in combination with obesity (0.95, 95% CI 0.87-1.0) or type 2 diabetes (0.95, 95% CI 0.90-1.00).

Conclusion: The ICD-10 code for NAFLD had a high PPV, that was further improved after exclusion of patients with coding for other liver diseases than NAFLD. This approach should be preferred when performing register-based studies to identify patients with NAFLD in Sweden.

P04-23-YI Growth and development of infants born to mothers with non-alcoholic fatty liver disease in pregnancy: a longitudinal study

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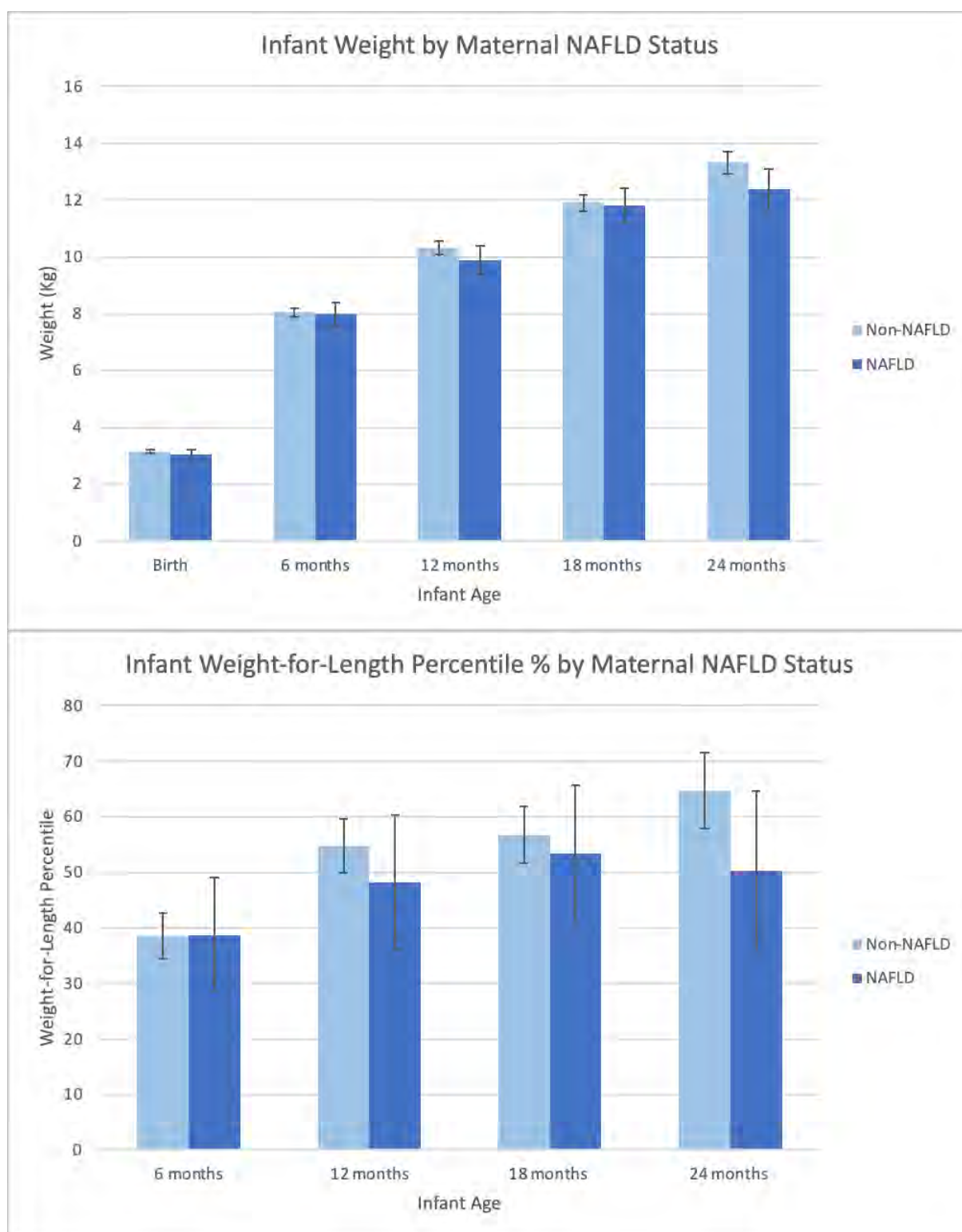
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Background and aims: Non-alcoholic fatty liver disease (NAFLD) affects 10-15% of pregnant patients in the United States. Several retrospective studies have shown that infants born to mothers with NAFLD have a higher risk of complications, including being born preterm and large for gestational age. However, there is limited data on continuing pediatric outcomes in children of mothers with NAFLD. We evaluated outcomes in infants born to mothers with NAFLD in pregnancy (compared to a control population of mothers without NAFLD) over the first 2 years of life.

Method: Subjects were identified through an ongoing prospective study of pregnant patients who were screened for NAFLD during obstetric care. We reviewed infant electronic health records for neonatal, 6 month, 12 month, 18 month, and 24 month outcomes. Primary outcomes were growth in the first 2 years of life, defined as weight and weight percentile at birth, 6 months, 12 months, 18 months, and 24 months. Secondary outcomes included Apgar score, rates of neonatal complications, and rates of developmental delay at each time point. Outcomes in infants of mothers with vs without NAFLD were compared using either a chi-square test or a two-tailed independent samples T test.

Results: We obtained neonatal data for 634 infants (13.7% maternal NAFLD), 6 month data for 225 infants (15.1% maternal NAFLD), 12 month data for 179 infants (13.4% maternal NAFLD), 18 month data for 173 infants (14.5% maternal NAFLD), and 24 month data for 89 infants (13.5% maternal NAFLD). Maternal NAFLD was positively associated with increased maternal BMI pre-pregnancy (31.1 vs 28.6, $p < 0.01$) and at delivery (35.6 vs 33.1, $p < 0.01$) and diabetes mellitus (11.5% vs 2.4%, $p < 0.01$). Maternal NAFLD was not associated with a significant difference in birth weight or weight-for-gestational-age percentile, or weight or weight-for-length percentile at 6 months, 12 months, 18 months, or 24 months (see figure). Maternal NAFLD was not associated with premature delivery < 37 weeks, low Apgar score, or NICU admission. However, maternal NAFLD was positively associated with very premature delivery < 32 weeks (6.9% vs 2.2%, $p = 0.010$) and increased incidence of neonatal death (2.3% vs 0.2%, $p = 0.01$). Maternal NAFLD was not associated with developmental delays at any of the time points.

Conclusion: There was an increased incidence of extreme premature delivery and neonatal death associated with maternal NAFLD, which should be validated in a larger study. Despite significant metabolic differences in mothers with vs without NAFLD, there was no measurable impact on pediatric growth or development in the first 2 years of life. No other adverse neonatal outcomes were found to be associated with maternal NAFLD. Further investigation is warranted to determine if any sub-cohorts of mothers with NAFLD (i.e. those with certain BMIs or more advanced liver disease) are associated with adverse pediatric outcomes.

Figure:

P04-24-YI The prevalence and the assessment of significant and advanced fibrosis of the liver by non-invasive tests in patients with type 2 diabetes mellitus

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Background and aims: The global prevalences of NAFLD (non-alcoholic fatty liver disease) and advanced fibrosis (AF) in patients with T2DM (type 2 diabetes mellitus) are 55.5% and 17.0% respectively. Non-invasive tests (NIT's) that detect patients at risk of AF are of paramount importance for the management of NAFLD.

This study aims to assess the prevalence of NAFLD and AF in a population with T2DM in two medical centers in Belgium [VITAZ, a non-university hospital in Sint-Niklaas and the University Hospital of Antwerp (UZA)]. Additionally, we studied the correlation between several biochemical NIT's and liver elastography (LE) in this patient population.

Method: The VITAZ cohort consisted of 320 ambulatory patients (169M/151F) that were recruited prospectively at the endocrinology department. For all patients over 35 years old with a diagnosis of T2DM for more than five years that did not have another underlying liver disease, the FIB-4 score was determined. In case of a high FIB-4 score, the patient was invited for an abdominal ultrasound (AU) with LE (acoustic radiation force impulse, Hitachi).

In the prospective UZA cohort, all T2DM patients hospitalized for yearly evaluation were examined, 272 (175M/97F) received a blood analysis, an AU and a LE (vibration-controlled transient elastography, Fibroscan®).

Results: In the VITAZ cohort, there was a prevalence of 6.88% (22/320) of probable AF based on FIB-4 scores. Of them, 78.75% (11/14) had a significant fibrosis (SF) based on LE [F2-F3 (5/11) and F4 (6/11)].

In the UZA cohort, 67.34% and 16.8% of patients had liver steatosis based on AU and SF on LE respectively. The correlation between the presence of SF on LE (≥ 7.9 kPa) and several NIT's (FIB-4, NFS, FLI) was examined [FIB-4 (AUC 0.644; OR 2.5; Cohens kappa 0.155); NFS (AUC 0.684; OR 1.4; Cohens kappa 0.033)], with the FIB-4 score being more sensitive and the NFS-score more specific (sensitivity 78.5% vs 24.4% and specificity 42.9% vs 90.5%). The fatty liver index (FLI) was a good predictor of liver steatosis.

Conclusion: The prevalence of AF in the VITAZ cohort based on FIB-4 scores is low. In the UZA cohort 57% of patients with a LE of ≥ 7.9 kPa, had a low FIB-4 (< 1.30). Probably, the 6.88% prevalence of AF in the VITAZ cohort is an underestimation because patients with SF on LE and a low FIB-4 score were missed. Although there was a good correlation between a high FIB-4 score and the presence of AF on LE (78.75%), patients with SF are missed with a risk stratification based on FIB-4 score alone; therefore, we recommend a LE in every T2DM at certain intervals.

The prevalence of NAFLD in the UZA cohort was very close to prevalence of NAFLD (68%) earlier reported in Europe. Here, SF on LE was present in 16.8% of patients. A similar percentage is described in literature, though related to AF. This underestimation is probably due to the exclusion of other fibrogenic risk factors in this study that can co-exist with NAFLD in real life.

P04-25-YI A screening strategy of non-alcoholic fatty liver disease in endocrine outpatients using the fibrosis-4 score.

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Background and aims: Liver fibrosis is the most important prognostic factor in Non-alcoholic fatty liver disease (NAFLD). Current clinical guidelines recommend to rule out the presence of advanced liver fibrosis (ALF) in high prevalence populations. Fibrosis-4 score (FIB-4) have been well validated with very good negative prognostic value in these contexts. Patients with insulin resistance, type 2 diabetes (T2D) and or metabolic risk factors are more prompt to develop NAFLD, thus, our laboratory implemented an automated screening strategy using FIB-4 in every outpatient attending endocrinology clinic in order to approach to the scope of probably NAFLD in our hospital.

Method: FIB-4 score was automatically added in all 3406 patients' analytics from endocrinology clinic of a tertiary hospital during 4 months. Cut-offs of <1.3 and <2 (low probability of ALF (LPF)) in patients aged >35 and >65 respectively, and >2.67 (high probability of ALF (HPF)) were applied. Intermediate values were considered undetermined (U). Patients were gathered into 4 categories based on diagnosis: T2D, type 1 diabetes (T1D), metabolic syndrome (MS) (at least 2 components) and others (mostly thyroid pathology). SPSS statistic was used for data analysis.

Results: 758 patients (22.25%) were classified as U or HPF. T2D and MS accounted for 56.29% and 86.5% of total U and HPF cases respectively.

Median of FIB-4 was significantly different based on patient's diagnosis. Age was the principal factor that conditioned the FIB4 distribution, which resulted significantly different between categories. None of the analytic parameters related to MS (total cholesterol, triglycerides, HDL cholesterol and glucose) showed significant correlation with FIB-4.

Conclusion: Based on FIB-4, almost a quarter of the patients attended endocrinology clinic would require special attention in order to prevent NAFLD progression. At least a 5% had a HPF and should be referred to hepatology clinic for further investigation. More than 50% of endocrine patients had high risk factors associated to NAFLD, so it is important to apply these kind of screening strategies with no added-costs in order to improve early diagnosis and therefore patient's prognosis.

Figure:

DIAGNOSIS	MEDIAN AGE (IQR)	N (%)*	MEDIAN FIB-4 (IQR)	LPF		U		HPF	
				N (%)	MEDIAN (IQR)	N (%)	MEDIAN (IQR)	N (%)	MEDIAN (IQR)
T2D	67 (16)	1371 (40.25)	1.29 (0.85)	1028 (74.9)	1.11 (0.57)	234 (17.1)	2.05 (0.75)	109 (8)	3.30 (1.07)
T1D	47 (21)	702 (20.61)	0.89 (0.6)	571 (81.3)	0.77 (0.63)	120 (17.1)	1.57 (0.63)	11 (1.6)	2.95 (0.29)
MS	59 (20)	459 (13.47)	1.15 (0.85)	324 (70.6)	0.93 (0.51)	97 (21.1)	1.64 (0.72)	38 (8.3)	3.00 (0.44)
OTHERS	52 (23)	874 (25.66)	0.97 (0.68)	725 (83)	0.86 (0.5)	137 (15.7)	1.63 (0.74)	12 (1.4)	3.11 (0.51)
TOTAL	59 (24)	3406	1.10 (0.78)	2648 (77.75)	0.94 (0.54)	588 (17.26)	1.76 (0.78)	170 (4.99)	3.18 (0.77)

Table. FIB-4 between diagnosis categories. *% of total cases. IQR (interquartile range).

P05-01 Impact of resmetirom-mediated reductions in liver volume and steatosis compared with placebo on the quantification of fibrosis using second harmonic generation in a serial liver biopsy study

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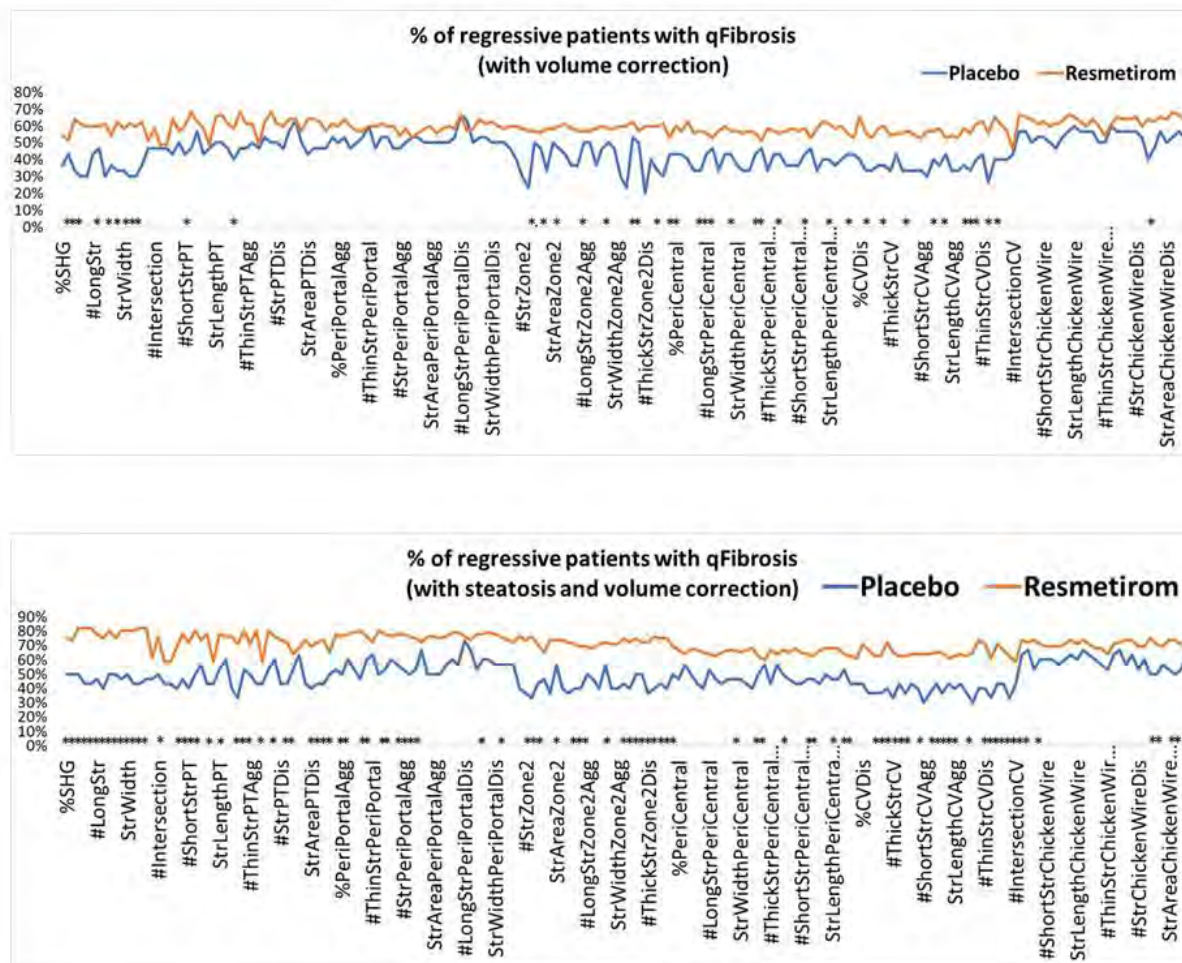
Background and aims: Resmetirom (MGL-3196) is a liver-targeted, oral, once-daily thyroid hormone receptor-beta selective agonist that reduces steatosis (by MRI-PDFF: 43% [80 mg]; 53% [100 mg], Phase 3) and liver volume (20%, Phase 2 serial liver biopsy study) that were elevated at baseline. Assessment of histological features that stage NASH fibrosis may be impacted in the setting of decreased steatosis and liver volume following therapeutic intervention. Artificial intelligence (AI)-based algorithms such as qFibrosis can incorporate normalization procedures to account for steatosis area and liver volume reduction, thereby improving detection of fibrosis changes. The aim of this analysis is to quantify and correct fibrosis changes related to resmetirom-mediated steatosis and liver volume changes not captured by the NASH Clinical Research Network (CRN) scoring system.

Method: Fibrosis was estimated as a continuous variable using second harmonic generation (qFibrosis)/two photon excited fluorescence for 102 paired biopsy samples from MGL-3196-05, a 36-week randomized double-blind, placebo-controlled Phase 2 serial liver biopsy study of resmetirom in adults with biopsy-confirmed NASH and $\geq 10\%$ hepatic fat based on MRI-PDFF (NCT02912260). qFibrosis (normalized by tissue area) was subsequently corrected for steatosis (tissue area-steatosis area) or liver volume reduction determined by PDFF (volume/average volume raw parameter based on all samples). In the final model, steatosis and liver volume were both corrected prior to assessment of qFibrosis. Relative changes in 184 fibrosis parameters were determined as (P) progressing ($\geq 10\%$), (R) regressing ($< 10\%$), or (N) no change.

Results: In normal qFibrosis without correction, resmetirom treatment resulted in a significant reduction in fibrosis (≥ 1 -point reduction) in F3 patients compared to placebo. With liver volume correction, 45/184 regression parameters demonstrated significant changes after resmetirom treatment (top panel). When steatosis and liver volume correction factors were combined, 111/184 ($p < 0.05$) regression parameters were reduced significantly with resmetirom treatment (bottom panel). Fibrosis regression was observed in all regions (portal, per-portal, zone 2, peri-central, central). Progression patterns were different from regression and also significantly impacted by correction.

Conclusion: Quantification of changes in NASH fibrosis are impacted by therapeutic interventions such as resmetirom that reduce liver volume. Correcting the AI-based algorithm of qFibrosis for liver volume and steatosis reveals the significant impact of resmetirom, regardless of NASH fibrosis stage, in the Phase 2 study.

Figure:



P05-02 Impact of bariatric surgery, gender, vitality and depressive symptoms on self-perceived health of obese patients with non-alcoholic fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is closely associated with obesity. Bariatric surgery has proven to be the most effective treatment for morbid obesity. NAFLD patients with obesity have been found to report an impaired quality of life. However, evidence of the influence of bariatric surgery on self-perceived health of these patients is contradictory, as is the impact of factors such as vitality, depressive symptoms, or gender. This study therefore aimed to find out whether vitality and depressive symptoms mediate the relationship between absence or presence of bariatric surgery and self-perceived health of NAFLD patients with obesity, and whether gender moderates this relationship.

Method: The sample of 162 biopsy-proven NAFLD patients with obesity was evaluated using the 12-Item Short-Form Health Survey (SF-12) and Beck Depression Inventory-II (BDI-II). The sample was divided into two groups, one with 81 patients (33 men and 48 women) who had undergone bariatric surgery, with a mean age of 50.02 years ($SD = 10.59$), and another group of 81 patients (33 men and 48 women) who had not undergone surgery, with a mean age of 50.74 years ($SD = 10.48$). Model 6 mediation was performed using the SPSS PROCESS v3.5 macro, while Model 91 was used for moderated mediation. In both models, 5000 bootstrap samples were employed to test the indirect effects estimated, which were considered significant when the confidence interval (CI) at 95% did not include 0.

Results: Vitality and depressive symptoms mediated the association between absence or presence of bariatric surgery and self-perceived health (effect = -4.627, CI = -7.410 to -2.389). The direct effect of passive/avoidance coping on self-efficacy was significant after mediation analysis (effect = -12.744, $p < 0.001$), showing partial mediation of vitality and depressive symptoms. Gender (beta = -0.129, $p = 0.002$) moderated the relationship between vitality and depressive symptoms. The indirect conditional effects of bariatric surgery on self-perceived health through vitality and depressive symptoms were higher in women than in men.

Conclusion: Bariatric surgery was associated with higher vitality and lower depressive symptoms, which in turn predicted better self-perceived health in NAFLD patients with obesity. In addition, the positive effects of bariatric surgery on the self-perceived health of these patients were higher in women than in men. This study therefore confirmed the positive impact of bariatric surgery on quality of life through its positive influence on patients' vitality and depressive symptoms. In conclusion, bariatric surgery is linked to a better biopsychosocial profile in NAFLD patients with obesity, especially in women.

P05-03-YI Advanced fibrosis in non-alcoholic fatty liver disease is independently associated with reduced renal function

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) and chronic kidney disease (CKD) are prevalent in the general population. NAFLD and CKD share several phenotypic characteristics, such as type 2 diabetes (T2DM), obesity and hypertension (HTN). Presence of metabolic syndrome, dysbiosis, and unhealthy diets have been postulated as mechanisms linking NAFLD and CKD. The aim of this study is to explore the association and risk factors between NAFLD and CKD in a cohort of patients with obesity, a population at high risk for developing both NAFLD and CKD.

Method: Patients with obesity (defined as body mass index [BMI] >30 kg/m²) and NAFLD (ICD-10 codes K76.0 and K75.8) were identified using electronic medical record (EMR) at a tertiary care clinic between 01/2019 to 01/2020. Demographics, ethnicity, medical conditions, markers of liver inflammation and synthetic function were recorded. To assess the health of renal function, glomerular filtration rates (GFR) were recorded. Fibrosis Index 4 (FIB4) was calculated to determine the degree of liver fibrosis. Univariate analysis was performed using t-tests for continuous variables and chi-square or Fisher's exact tests for categorical variables. Multivariate analysis and adjustment for confounders was performed using generalized linear models including linear regression.

Results: A total of 546 patients with NAFLD were identified from a cohort of patients with obesity. Majority were female (58.6%), mean age 56 years (SD 12.7), and mean BMI 35.7 kg/m² (SD 5.49). Hispanics constituted 24%, 15.2% were black and 37.9% were white. Approximately half (49.6%) had HTN and 34.9% had T2DM. Age ($p < 0.001$), ALT ($p = 0.01$), albumin ($p = 0.011$) and FIB4 ($p = 0.005$) were associated with changes in GFR in univariate analysis. In univariate linear regression, reducing GFR was associated with higher FIB4 (effect size [beta] of one-unit increase in GFR on FIB4 = -0.013, $p < 0.001$). In multivariate linear regression, T2DM was independently associated with increased liver fibrosis (effect size of T2DM on FIB4 = 0.387925, $p < 0.02$). After adjustment for T2DM, the relationship between GFR and FIB4 was maintained (beta -0.012, $p < 0.001$). HTN did not significantly change the relationship between GFR and FIB4 in this model ($p > 0.5$). When compared to white race, only patients of black race had a lower FIB4 (effect size of black race on FIB4 = -0.442 compared to white race, $p < 0.05$). Adjusting for race did not alter the underlying relationship between GFR and FIB4.

Conclusion: NAFLD is independently associated with reduced renal function in obese patients. Black race was associated with lower FIB4. In our analysis, we show an inverse relationship between liver fibrosis and GFR which needs to be further explored.

Figure:

Univariate Analysis							Multivariate Analysis		
	GFR \geq 90 mL/min (n=232)	GFR 60-89.99 mL/min (n=240)	GFR 45-59.99 mL/min (n=46)	GFR 30-49.99 mL/min (n=7)	GFR <30 mL/min (n=4)	P value		Estimate (beta)	P value
Age in years (mean (SD))	51.9 (12.3)	57.1 (12.1)	66.9 (7.7)	69.7 (5.1)	69.3 (8.9)	<0.001	GFR	-0.0122	<0.001
Sex: Male (N (%)) Female (N (%))	89 (38.4%) 143 (61.6%)	109 (45.5%) 131 (54.5%)	19 (41.3%) 27 (58.7)	1 (14.3%) 6 (85.7%)	3 (75%) 1 (25%)	0.179	HTN	0.0305	0.8468
Ethnicity: Hispanic (N (%)) Non-Hispanic (N (%)) Unknown (N (%))	58 (25%) 102 (44%) 72 (31%)	59 (24.6%) 116 (48.3%) 65 (27.1)	8 (17.4%) 26 (56.5%) 12 (26.1%)	0 7 (100%) 0	1 (25%) 2 (50%) 1 (25%)	N/A	T2DM	0.3899	0.0164
AST (IU/L) (mean (SD))	39.89 (36.6)	35.24 (29.3)	32.02 (25.1)	23.57 (9.1)	23.75 (15.4)	0.238	White Race	Reference	NA
ALT (IU/L) (mean (SD))	50.88 (54.7)	42.71 (42.7)	29.09 (19.9)	19.29 (7.1)	13.75 (5.1)	0.01	Black Race	-0.4415	0.0465
Albumin (mean (SD))	4.07 (0.5)	4.11 (0.5)	3.95 (0.5)	3.54 (0.4)	4.00 (0.6)	0.011	Asian Race	-0.4712	0.3459
Platelet count \times $10^9/L$ (mean (SD))	243.70 (71.4)	232.63 (70.2)	213.85 (67.2)	242.71 (104.6)	208.00 (99.6)	0.08	Other Race	0.0079	0.9664
Mean FIB4	1.49 (1.9)	1.57 (1.1)	2.26 (2.4)	2.14 (1.84)	3.67 (4.8)	0.005	Unknown Race	-0.0065	0.9752

P05-05 Community NAFLD screening programme in patients with T2DM indicates high burden of undiagnosed liver disease

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Background and aims: NAFLD is the most common liver disease in Western countries, affecting 1 in 4 adults. Hepatic fibrosis is an important predictor of liver-related morbidity and premature mortality. Vibration-controlled transient elastography (VCTE) is a validated non-invasive test with NPV >90% for detection of advanced fibrosis/cirrhosis but largely confined to specialist centres.

Method: To assess the feasibility of VCTE as a screening method to detect hepatic fibrosis in patients with risk factors for NAFLD in a community healthcare setting. 206 patients (112 female-94 male) with risk factors for NAFLD were identified via dispensing records in pharmacies and invited to register. VCTE assessments were performed in 4 pharmacies between June-July 2021 and results sent to patients' GPs. Patients with liver stiffness measurements (LSM) >8.2 kPa were referred to a specialist Hepatology clinic.

Results: The median age was 63 years (range 27-84). 88% of patients had T2DM and 53% had BMI ≥ 30 . The median CAP was 291dB/m (range 160-400) and 45% of patients had CAP ≥ 300 dB/m. The median LSM was 5.8kPa, and 31% patients had LSM ≥ 7.1 kPa. 12% had LSM ≥ 9.7 kPa, 6.7% had LSM ≥ 12.5 kPa. Only 19% of patients had both a normal CAP and LSM.

Conclusion: This is the first study to assess VCTE screening for hepatic fibrosis beyond traditional healthcare settings, and demonstrates that community-based risk-stratified screening leads to earlier identification of patients with liver fibrosis. Community pharmacy is an accessible healthcare setting in which access to non-invasive assessments of hepatic fibrosis outside of the hospital setting could be offered with potential cost savings to the healthcare system.

P05-06 Prevalence and clinical characteristics of non-alcoholic fatty liver disease in morbidly obese patients before and after bariatric surgery

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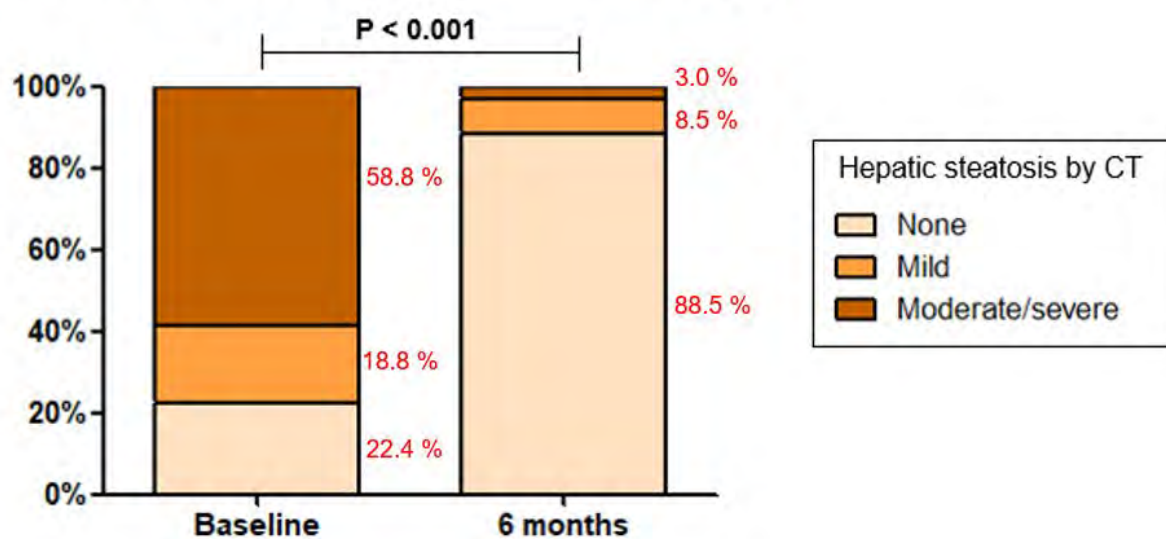
Background and aims: The aim of this study is to investigate the prevalence and clinical characteristics of non-alcoholic fatty liver disease (NAFLD) in morbidly obese patients who underwent bariatric surgery, and to compare liver and visceral fat before and after bariatric surgery using non-contrast computed tomography (CT) images, which was validated with liver pathology.

Method: The prevalence of NAFLD was estimated by CT image, and clinical characteristics between NAFLD and non-NAFLD groups were compared. There were 165 patients who had paired CT images at pre-operation within 1 month and post-operative 6 months. In these patients, changes in liver, visceral, and subcutaneous fat were compared after surgery.

Results: In the intraoperative liver biopsy subgroup analysis (n = 21), the hepatic steatosis on pathology and CT was 90.5% (20/21) and 85.7% (18/21), respectively, showing a high concordance rate. In addition, NAFLD activity score ≥ 5 (NASH) was found in 42.9%, and $\geq F3$ fibrosis in 23.8%. At pre-operation, 209 morbidly obese patients showed a mean age of 38.9 years, a 63.2% female proportion, and a median body mass index (BMI) of 38.0 kg/m². The frequency of hypertension (53.6%), diabetes (35.4%), and hyperlipidemia (37.8%) was noticed. The median pre-operative AST and ALT levels were 35 and 51 IU/L. Among them, the prevalence of NAFLD was 81.3%, those showed younger age (38.3 vs 41.2 years), lower female proportion (58.2% vs 84.6%), and higher levels of BMI (38.7 vs 36.6), ALT (56.0 vs 24.0 IU/L), GGT (33.0 vs 17.0 IU/L), APRI score (0.4 vs 0.3) and visceral adiposity index (148.4 vs 118.6 cm²/m²) than non-NAFLD patients. At 6 months post-operation (n = 165), median BMI decreased from 38.0 to 28.5 kg/m² (p < 0.001), ALT level decreased from 51.0 IU/L to 15.0 IU/L, and prevalence of NAFLD decreased from 77.6% to 11.5%.

Conclusion: The prevalence of NAFLD in the morbidly obese patients was 80%, but it decreased 12% after 6 months of bariatric surgery with improvement of liver enzyme profiles. Liver fat assessment using CT showed a higher concordance rate with liver biopsy, and the existence of a considerable proportion of NASH and advanced fibrosis favors performing liver biopsy in these morbidly obese patients during bariatric surgery.

Figure:

Evolution of NAFLD after bariatric surgery

P05-09 Fluorescent advanced glycation end products levels in the brazilian longitudinal study of adult health (ELSA-Brasil): a potential biomarker for risk stratification of non-alcoholic fatty liver disease-associated steatosis

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Background and aims: Liver diseases are associated with the excess formation of advanced glycation end-products (AGEs), which induce tissue inflammation and oxidative damage. However, the trend of oxidative marker levels according to the steatosis grade in NAFLD is unclear. For this purpose, serum AGE levels were compared between participants with NAFLD accordingly to steatosis severity in the baseline ELSA-Brasil population.

Method: The participants (n = 305) were grouped according to the severity of steatosis: mild and moderate/severe pooled, classified by ultrasound hepatic attenuation. The measurement of serum fluorescent AGE concentrations was based on spectrofluorimetric detection. Serum AGE content and clinical and laboratory characteristics of the participants were compared between groups. Logistic regression analysis was used to investigate the relationship between serum AGE levels and steatosis severity.

Results: According to the steatosis severity spectrum in NAFLD, individuals with the most severe steatosis grade had a higher incidence of metabolic syndrome (63% vs 34%, $p \leq 0.001$), diabetes mellitus (37% vs 14%, $p \leq 0.001$), and high cholesterol levels (51% vs 33%, $p < .001$). Moreover, individuals with increasing severity of steatosis presented increasing waist circumference, BMI, systolic and diastolic blood pressure, fasting blood glucose, glycated hemoglobin, insulin, triglycerides, ALT, GGT, C-reactive protein, and uric acid levels and lower HDL. Higher serum AGE content was present in the moderate/severe group of individuals than in the mild group ($p = 0.008$). In addition, the serum AGE levels were correlated with the steatosis grade in the overall sample ($\rho = 0.146$, $p = 0.010$). Logistic regression analysis, after adjusting for confounding variables, showed that subjects with higher serum AGE content had a 4.6-fold increased chance of having moderate or severe steatosis when compared to low levels of serum AGEs. According to the results of the ROC analyses (AUC = 0.83), AGEs could be a good marker of steatosis severity in patients with NAFLD, strengthening the involvement of AGE in NAFLD pathogenesis.

Conclusion: Therefore, serum fluorescent AGE quantification by spectroscopy could be a promising alternative method to monitor progression from mild to severe NAFLD accordingly to steatosis grade.

P05-10 Multiparametric ultrasound evaluation of the liver: whom would mostly benefit?

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Background and aims: The non-invasive multiparametric assessment of liver fibrosis, steatosis and inflammation offers valuable prognostic information in chronic liver disease (CLD) patients. The current study aims to evaluate and compare the feasibility and performance of three new ultrasound-based techniques for the non-invasive assessment of liver fibrosis, steatosis and inflammation in patients with alcohol related (ALD), viral related or metabolic associated fatty liver disease (MAFLD).

Method: 178-consecutive compensated CLD patients were included in the study. Ultrasound-based measurements were performed in all patients, in the same session, using ShearWave Elastography (2D-SWE.PLUS), Attenuation Plane-wave Ultrasound (Att.PLUS), Viscosity Plane-wave Ultrasound (Vi.PLUS) from Aixplorer and Transient Elastography (TE) with Controlled Attenuation Parameter (CAP) from Fibroscan as reference method.

Results: Valid measurements were obtained in 99% of patients by TE, in 90% of patients by 2D-SWE.PLUS/Vi.PLUS, and in 95.5% of patients by Att.PLUS. Irrespective of etiology, TE values showed a strong correlation with 2D-SWE.PLUS values ($R = 0.82$ - >0.89). In ALD and viral group, TE correlated best with 2D-SWE.PLUS values, followed by NFS and Fib4 values, while in MAFLD group the best correlation was with NFS ($R = 0.9$), similar to 2D-SWE.PLUS and followed by eLIFT and Fib4 ($R = 0.89$, $R = 0.87$, $R = 0.78$). Att.PLUS had a moderate performance in predicting different steatosis stages, with best results in MAFLD (AUC of 0.75 - >0.86), followed by viral (AUC of 0.75 - >0.8) and ALD (AUC of 0.66 - >0.7). Viscosity had excellent performance for predicting significant fibrosis, with best results in ALD and MAFLD (AUC of 0.9) but with a less predictive value than 2D-SWE.PLUS (AUC of 0.93 to 0.99). Viscosity correlated poorly with platelet-to-lymphocytes ratio in ALD group and with GPT and neutrophil-to-lymphocyte ratio in viral group, but with no inflammatory parameter in MAFLD.

Conclusion: The multiparametric ultrasound evaluation offers valuable prognostic information in a single analysis in CLD patients irrespective of etiology. 2D-SWE.PLUS has excellent diagnostic accuracy of liver fibrosis with similar value with serologic non-invasive markers in MAFLD subgroup. Att.PLUS has a relatively good accuracy of liver steatosis, while viscosity proved to better reflect liver fibrosis stage than liver inflammation, especially in MAFLD patients.

P05-12 Quantification of hepatic steatosis in patient with non-alcoholic fatty liver disease: comparison of sound speed, attenuation coefficient and continuous CAP measurements with MR-PDFF

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is becoming a major health problem, resulting in hepatic, metabolic and cardio-vascular morbidity. Our study aims at evaluating new ultrasonographic tools to detect and measure hepatic steatosis.

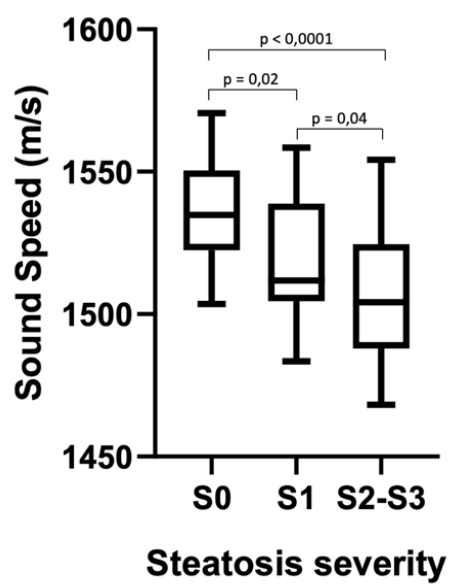
Method: After local approval and patient consent, we included 73 patients addressed to a hepatology consultation for NAFLD suspicion or follow-up. They underwent ultrasonographic measurement of liver sound speed estimation (SSE) and attenuation coefficient (AC) using Aixplorer MACH 30 (Supersonic Imagine, France), and continuous Controlled Attenuation Parameter (cCAP) using Fibroscan (Echosens, France). Hepatic steatosis was then classified according to MRI proton density fat fraction (PDFF). Receiver operating curve (ROC) analysis was performed to evaluate the diagnostic performance in the diagnosis of steatosis.

Results: Our patients had high mean BMI, weight, and waist circumference. 67 % had a metabolic syndrome and 15 % significant liver fibrosis. According to PDFF, 18 (24.7 %) patients had no steatosis (PDFF <6.5 %, S0), 30 (41.1 %) suffered from mild steatosis (6.5 <PDFF <16.5 %, S1), 8 (10.9 %) from moderate steatosis (16.5 <PDFF <22 %, S2), and 17 (23.3 %) from severe steatosis (PDFF >22 %, S3). SSE, AC and cCAP correlate with PDFF, with respective Spearman correlation coefficient at -0.50, 0.51 and 0.65 ($p < 0.01$). Mean SSE, AC and cCAP values significantly differ between non-steatotic and steatotic patients (S1-S3). SSE values also differs between S1 and S2-S3 steatosis ($p = 0.04$). An SSE threshold of 1524 m/s had a sensitivity of 69 % and a specificity of 74 % in the diagnosis of steatosis (S1-S3). The corresponding area under the ROC curve (AUC) was 0.77 (95 % CI 0.66-0.88, $p < 0.01$). An AC threshold of 0.41 dB/cm/MHz had a sensitivity of 91 % and a specificity of 79 % in the diagnosis of steatosis (S1-S3). The corresponding AUC was 0.91 (95 % CI 0.84-0.98, $p < 0.01$). An cCAP threshold of 263 dB/m had a sensitivity of 82 % and a specificity of 78 % in the diagnosis of steatosis (S1-S3). The corresponding AUC was 0.92 (95 % CI 0.86-0.98, $p < 0.01$).

Conclusion: SSE and AC, simultaneously measured using Aixplorer MACH 30 system, demonstrate their reliability to detect liver steatosis. AC has high sensitivity for all-grade steatosis detection, with AUC >0.90, despite having more than 40 % of our patients suffering from mild steatosis (S1).

To be noted that mean SSE values significantly differ between S1 and S2-S3 patients, maybe allowing for future ultrasonographic steatosis grading. cCAP has good overall performances, but optimal thresholds are yet to be determined.

Figure:



P05-13-YI Clinical variables influence performance of non-invasive tests for non-alcoholic fatty liver disease

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Background and aims: Non-invasive liver tests (NITs) for staging fibrosis in patients with non-alcoholic fatty liver disease (NAFLD) are increasingly being adopted in the clinic. Yet the performance of these tests may be influenced by clinical factors. Here, we aimed to evaluate whether age, sex, body mass index (BMI) and type 2 diabetes mellitus (T2DM) affect the performance of NITs in detecting advanced fibrosis in patients with NAFLD.

Method: A total of 966 adult patients of the LITMUS metacohort with histologically characterized NAFLD were included and were categorized based on age, sex, BMI, and T2DM. The diagnostic performance of Fibrosis-4 Index (FIB4) and Enhanced Liver Fibrosis (ELF) and vibration controlled transient elastography liver stiffness (VCTE LSM) were evaluated in different subsets of patients where the tests' results were available. Accuracy was expressed as the area under the ROC curve (AUC). Thresholds for diagnosis of advanced fibrosis ($\geq F3$) were calculated for each NIT for fixed (high) sensitivity and specificity.

Results: Differences in AUC levels between male and female, age categories, BMI categories and patients with and without T2DM tended to be small for all three tests, indicating comparable performance in detecting advanced fibrosis irrespective of these clinical factors. However, different thresholds were needed to achieve the same level of accuracy for each test. For example, to get 90% spec in patients with BMI >30 , LSM threshold for advanced fibrosis had to be 15.3kPa, much higher than the threshold for non-overweight participants (BMI <26): 10.5kPa. With a fixed sensitivity the thresholds for all three NITs were higher in patients with T2DM and also with increasing BMI.

Conclusion: Clinical characteristics of patients with NAFLD affect performance levels of NITs in detecting advanced liver fibrosis. This notion should be kept in mind using NITs in clinical practice. Large prospective studies estimating covariate-adjusted ROC curves are called for to define good accuracy levels of NITs in different patient settings, when taking effects of multiple clinical characteristics into account.

Figure: Thresholds of the tests to detect advanced liver fibrosis in different subgroups with NAFLD to achieve fixed sensitivities and specificities

	ELF			FIB4			VCTE LSM		
	n	Sensitivity 90%	Specificity 90%	n	Sensitivity 90%	Specificity 90%	n	Sensitivity 90%	Specificity 90%
Female	377	8.75	10.32	558	0.87	1.86	396	7.05	13.40
Male	529	8.62	10.01	395	0.83	2.05	230	7.65	14.05
Age 18-45	271	6.23	9.68	282	0.41	1.17	168	7.55	11.85
Age 45-60	387	8.67	10.06	411	0.95	1.83	272	6.25	14.05
Age > 60	248	9.10	10.77	260	1.10	2.76	186	5.95	14.20
BMI < 26	76	6.91	9.97	78	0.54	1.86	72	5.00	10.45
BMI 26-30	264	8.54	9.98	277	0.75	2.24	224	6.75	11.95
BMI > 30	566	8.75	10.22	598	0.86	1.80	330	7.95	15.25
DM	378	8.75	10.36	405	0.87	2.19	584	7.85	16.40
non-DM	528	7.04	10.02	548	0.58	1.80	342	7.05	11.95

P05-16 Association of thyroid dysfunction and raised sgpt values in children with obesity and severe obesity in greece: results of a tertiary paediatric hospital experience

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Background and aims: Paediatric obesity is a serious public health issue. The aim of this study was to explore whether SGPT values as proxy of possible liver injury differ in relation to FT4, TSH, HOMA-IR and Tanner stage in obese and severely obese children and adolescents.

Method: This is a retrospective study of Greek children/adolescents who attended outpatient clinics in a tertiary children's hospital over a ten-year period. 279 children (51.3% females) with BMI \geq 95th percentile according to CDC BMI curves were divided in two groups: Group 1, obese with 95th percentile \leq BMI <99th percentile, Group 2, severely obese with BMI \geq 99th percentile. Age, gender, Tanner stage prepubertal vs pubertal (stage 1 vs 2), BMI (Kg/m²), SGPT (mg/dl), TSH (mIU/ml), FT4 (ng/dl) levels and insulin resistance (defined as HOMA-IR \geq 2.5) were recorded. Mann Whitney test was used to compare above parameters between the groups. Liver injury was considered if SGPT values >22 mg/dl (females) and >26 mg/dl (males) according to the SAFETY study¹. Thyroid dysfunction was defined as FT4 <1 ng/dl. Pearson's χ^2 test was used to evaluate the association between categorical variables. Multiple linear regression model was applied in order to predict SGPT values, using gender, age, TSH, FT4, HOMA-IR categories, Tanner 1 and 2 and obesity vs severe obesity as explanatory co-variables. SPSS software version 26 was used.

Results: Children with severe obesity (group 2 with median BMI 29.3 IQR 6.0, $p < 0.001$), were significantly younger (median age 9, 9 IQR 5, 4, $p = 0.003$) and had significantly higher SGPT levels (median value 21.0 IQR 11.0, $p = 0.001$), compared with obese children from group 1. Higher frequency of raised SGPT as biomarker of liver injury was noted in children with FT4 <1 ng/dl, compared to children with FT4 ≥ 1 (53.3% vs 25%, $p = 0.024$). There was significantly higher frequency of raised SGPT in severely obese subjects compared to obese subjects (40.4% vs 23.1%, $p = 0.003$). According to multiple linear regression model for predicting SGPT values, we found that age increase had a significantly positive effect on SGPT (coef.: 1.14 per one year increase, 95% C.I. 0.08 2.20, $p = 0.035$), while FT4 ≥ 1 vs FT4 <1 groups (coef.: -12.98, 95% C.I. -21.35 -4.62, $p = 0.003$) and Tanner 2 vs 1 (coef.: -7.46, 95% C.I. -13.72 -1.20, $p = 0.020$) were inversely associated with SGPT. There were no statistically significant effects of gender, TSH, HOMA-IR, obesity status on SGPT values.

Conclusion: Severely obese Greek children and adolescents who attended outpatient clinics over the last ten years have increased SGPT values in comparison to obese peers. SGPT values were significantly affected by increasing age, lower FT4 values and Tanner stage.

Reference: ¹ Schwimmer JB et al SAFETY study: alanine aminotransferase cut off values are set too high for reliable detection of paediatric chronic liver disease. *Gastroenterology*. 2010 Apr;138 (4):1357-64.

P05-20-YI Performance of FIB-4 compared to vibration controlled transient elastography in people with type 1 and type 2 diabetes

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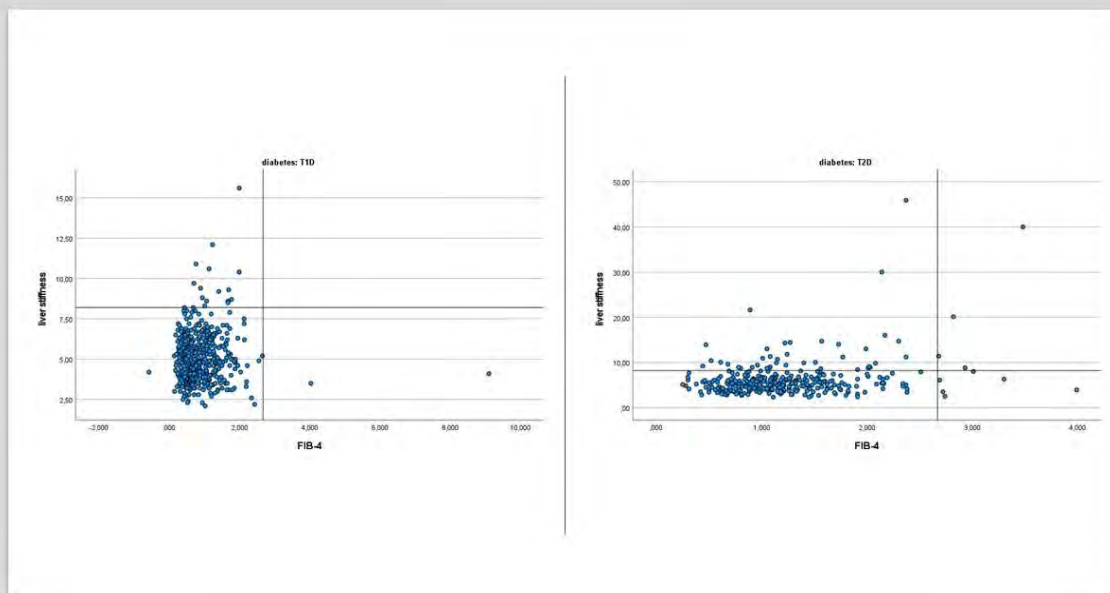
Background and aims: Risk scores are proposed to screen for significant fibrosis in NAFLD suspects, but their accuracy in real-world settings is debated. Transient elastometry (TE) is currently considered the method of reference, therefore this study aims to evaluate the accuracy of FIB-4 compared to TE to predict significant fibrosis in a cohort of patients with type 1 (T1D) or type 2 (T2D) diabetes.

Method: This was a cross-sectional sub-analysis of data on subjects with T1D or T2D that were consecutively screened for fatty liver disease with ultrasound and TE (Fibroscan®, Echosens, France), in a Belgian tertiary care center (NCT04664036). All subjects had a diabetes duration ≥ 1 year. For the present study, we included subjects with at least 10 successful acquisitions and an interquartile range/median < 30 %, who were free from secondary causes of steatosis or chronic liver disease. FIB-4 was calculated as appropriate using age-specific cut-offs. A liver stiffness measurement (LSM) ≥ 8.2 kPa was considered significant fibrosis ($\geq F2$).

Results: A total of 863 subjects were included, 529 had T1D, 334 had T2D. Based on ultrasound or controlled attenuation parameter ≥ 274 dB/m, NAFLD was present in 21.4 % of people with T1D, and 71.9 % of people with T2D. Significant fibrosis based on TE was present in 3.2% of subjects with T1D, and 13.8 % of subjects with T2D. According to FIB-4, 0.4 % of people with T1D had significant fibrosis, and 3.0 % in people with T2D. The correlation between FIB-4 and LSM was weak in those with T2D (0.291, $p < 0.001$), and not significant in people with T1D. AUROC of FIB-4 in people with T1D was 0.74, $p = 0.001$, and 0.64, $p = 0.004$ in people with T2D, the shapes of the curves clearly indicated that general cut-off points could not be applied. Sensitivity of FIB-4 > 2.67 to rule in fibrosis in people with T2D was 70%, specificity was 79%. Gender-based stratification did not alter the AUROC significantly in neither type of diabetes. Interestingly, waist circumference, stratified per sex, was an adequate predictor of significant fibrosis in people with T2D (AUROC for males: 0.81, $p < 0.001$; AUROC for females: 0.71, $p < 0.001$), but not for people with T1D (AUROC for males: 0.58, $p = 0.412$; AUROC for females: 0.54, $p = 0.711$), which might be attributable to lack of power due to the small number of cases.

Conclusion: Significant fibrosis is common in people with NAFLD and T2D, but not in those with NAFLD and T1D. FIB-4 correlates poorly with LSM and the optimal cut-off to rule-in fibrosis is difficult to define. Waist circumference is a potential surrogate index for liver fibrosis. More data on fibrosis in T1D is needed.

Figure: Scatterplot of FIB-4 versus LSM in T1D (left) and T2D (right). Cut-offs (FIB-4: 2.67 and VCTE: 8.2 kPa) are shown using vertical and horizontal lines.



P05-24 VEGF and TGF- β 1 in children with non-alcoholic fatty liver fibrosis

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Background and aims: To investigate the differences in vascular endothelial growth factors (VEGF) and transforming growth factor beta1 (TGF- β 1) levels in children with non-alcoholic fatty liver disease (NAFLD) depending on the liver fibrosis presence, to study its correlations with non-invasive fibrosis grade.

Method: The study included 50 patients aged 9 to 17 years (average age 12.15 ± 2.51 years). According to body mass index (BMI), and transient elastography (Fibroscan@502 touch, Echosens, France) data patients were divided into 4 groups: 1 group consisting of 13 patients with NAFLD and liver fibrosis \geq F1 (METAVIR), 2 group-of 16 patients with NAFLD without fibrosis, 3 group-11 obese patients without liver steatosis and fibrosis, 4 group (control)-10 patients with normal weight without liver steatosis and fibrosis. Serum VEGF, IL-8 levels (Wuhan Fine Biotech Co., Ltd, China), and TGF- β 1 levels (IBL International, Germany) were determined by the enzyme-linked immunoassay. Anthropometry with BMI calculation was conducted.

Results: The mean value of VEGF in children with NAFLD and fibrosis was higher 2.0 times ($p < 0.05$) compared to the control group children. The median VEGF level in patients with NAFLD and fibrosis was higher at 1.3 times ($p > 0.05$) and 1.2 times ($p > 0.05$) than in 2 and 3 group patients, respectively. The mean TGF- β 1 level in patients with NAFLD and fibrosis was higher at 1.5 times ($p < 0.05$), 1.5 times ($p < 0.05$), and 1.3 times ($p > 0.05$) than in the control group, 2 and 3 groups, respectively. Positive correlation between VEGF level and liver fibrosis grade (METAVIR) ($r = 0.372$; $p = 0.036$), IL-8 ($r = 0.443$; $p = 0.014$) was found.

Conclusion: Thus, in children with non-alcoholic fatty liver fibrosis, a significant increase in the VEGF and TGF- β 1 mean values which correlated with non-invasive liver fibrosis grade was found. Therefore, VEGF and TGF- β 1 levels can be useful as non-invasive liver fibrosis markers in children with NAFLD that might help select patients for active therapeutic intervention.

P05-25 Urinary lithogenic profile in patients with non-alcoholic fatty liver disease (NAFLD)

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Background and aims: Several epidemiological studies observed an increased risk for nephrolithiasis in patients affected by non-alcoholic fatty liver disease (NAFLD). However, the urinary lithogenic risk profile for patients with NAFLD is currently unknown.

The aim of this study is to assess the urinary metabolic profile of patients with NAFLD in different stages of disease, compared with a group of healthy subjects.

Method: We systematically analyzed the urinary metabolic profile of patients affected by NAFLD and compensated liver cirrhosis (LC), followed at the Hepatology outpatient clinic of our Institution, compared to healthy individuals. A complete metabolic work-up for kidney stone disease was performed in all subjects. Multivariable linear regression models adjusted for age, sex, body mass index, eGFR and diabetes, were applied to assess differences in urinary lithogenic risk factors between healthy individuals and patients affected by NAFLD and LC.

Results: A total of 42 patients and 12 healthy individuals were included in the analysis. No differences in age, sex and BMI were found between patients affected by NAFLD and LC, whereas the latter were more diabetic (71.4% vs 14.3%). After adjusting for multiple confounders (eGFR, age, gender, BMI) patients with NAFLD and LC had lower urinary magnesium (β -55; 95% CI -104, -6; p = 0.03 and β -63; 95%CI -121, -6; p = 0.03, respectively) and lower oxalate (β -23.4; 95% CI -44.1, -2.7; p = 0.05 and β -22.1; 95%CI -45.7, 1.5; p = 0.06, respectively) excretions, compared with healthy controls. Compared with NAFLD patients, LC patients showed higher urinary ammonia (β 31.7; 95% CI 14.4, 48.9; p <0.01), ammonia/net acid excretion ratio (β 0.4; 95% CI 0.2, 0.6; p <0.01), urine pH (β 1.16; 95% CI 0.44, 1.88; p = 0.01) and lower urinary uric acid excretion (β -210; 95% CI -419.8, -0.3; p = 0.05) and titratable acidity (β -9.7; 95% CI -19.0, -0.4; p = 0.04).

Conclusion: Altered urinary ammonia and magnesium excretions seems to be involved in the higher risk for stone formation found in patients with NAFLD. More studies are needed to investigate on stone phenotypes and renal handling of ammonia in this setting.

P06-01 High yield of systematic hepatitis B virus screening among migrants in Amsterdam, the Netherlands with increased risk for non-alcoholic fatty liver disease

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Background and aims: An unknown but likely substantial part of individuals with chronic hepatitis B (HBV) or hepatitis C (HCV) in the Netherlands are unidentified, with migrant groups harbouring most undiagnosed individuals. Aiming to increase viral hepatitis screening yield, we report on HBV and HCV screening within people at risk for non-alcoholic fatty liver disease (NAFLD) from a multi-ethnic cohort.

Method: As part of the NILE study, which assessed non-alcoholic fatty liver disease (NAFLD) in the general population in Amsterdam, the Netherlands, six ethnic groups were recruited. Participants had elevated fibrosis non-invasive liver tests (NITs) (fibrosis-4 index (FIB4) or AST to platelet ratio index (APRI)) and/or metabolic risk factors (T2DM, BMI $\geq 30\text{kg/m}^2$, waist-hip-ratio ≥ 0.90 or fatty liver index (FLI) ≥ 30), or were controls. In all, transient elastography (TE) and anti-HBc, HBsAg and anti-HCV were assessed. HCV-RNA was determined in those anti-HCV positive. Two targeted screening approaches-selection based on elevated fibrosis NITs or on metabolic risk factors-were compared with a generic screening approach-without a priori selection on risk of liver disease. Based on earlier data from this multi-ethnic population, participants were divided in a low and intermediate endemic population based on HBsAg prevalence.

Results: We included 346 participants with elevated NAFLD risk and 57 controls, most with a Dutch (n = 103, 26%), South-Asian Surinamese (n = 91, 23%), African Surinamese (n = 89, 22%), or Ghanaian (n = 63, 16%) ethnic background, 84% were first generation migrants. Two individuals from Ghana were anti-HCV positive yet HCV-RNA negative. For HBV, 86 (21%) were anti-HBc positive, of whom 11 (3%) HBsAg-positive. Of these, 10 (91%) were unaware of the infection or lost to follow-up. The highest anti-HBc and HBsAg prevalence was observed in Ghanaian participants (n = 40, 64% and n = 4, 6%, respectively). HBsAg-positive participants (n = 11) had lower TE controlled attenuation parameter (CAP) as measurement for liver steatosis, compared to HBsAg-negative participants (n = 75) with a median of 239dB/m (IQR 227-269) and 253dB/m (IQR 213-297), respectively. Figure 1 shows the screening approaches in different groups. For the intermediate HBsAg-prevalence group (Ghanaian, Turkish and African Surinamese), HBsAg prevalence was $\geq 3.5\%$ for all screening strategies. For the low prevalence group (South-Asian Surinamese and Moroccans), HBsAg prevalence was 0.8% with generic screening and $\geq 3.9\%$ in the targeted screening groups.

Conclusion: Among migrants with elevated NAFLD risk from low HBsAg prevalence countries, targeted screening based on metabolic risk factors can improve HBV screening yield.

Figure: HBV serology results in the different subgroups of the NILE study in Amsterdam, the Netherlands, stratified by HBsAg prevalence of ethnic background

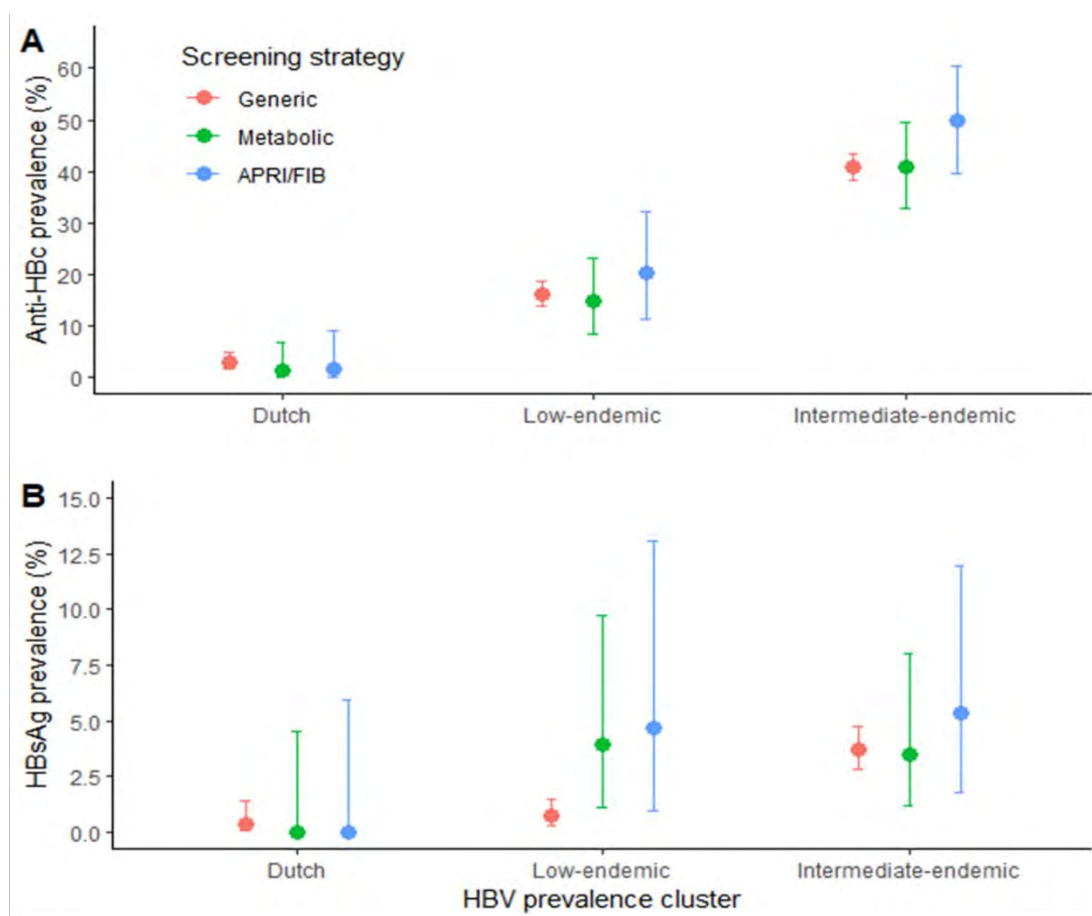


Figure legend: The low-endemic hepatitis B virus (HBV) prevalence cluster included participants with a Moroccan or South-Asian Surinamese ethnic background. The intermediate-endemic HBV prevalence cluster included participants with a Ghanaian, Turkish or African Surinamese ethnic background.

P06-02-YI Pancreatic T1 values on magnetic resonance imaging are correlated with portal inflammation scores on liver biopsy in patients with non-alcoholic fatty liver disease

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Background and aims: In obesity and metabolic syndrome, the pancreas can become infiltrated with adipocytes. Whether pancreatic steatosis causes inflammation and fibrosis and leads to organ dysfunction or malignancy, similar to liver damage occurring in Non-Alcoholic Fatty Liver Disease (NAFLD), is yet unclear. Both have overlapping risk factors, but their relationship is poorly explored. We have previously shown that multiparametric MRI parameters of the liver (proton density fat fraction (PDFF) and T1 mapping, typically associated to fibro-inflammation) strongly correlate with liver histology scores. The aim of the current study was to assess pancreatic steatosis and fibro-inflammation in individuals with proven NAFLD, and to evaluate whether pancreatic outcomes correlate with disease severity on liver histology.

Method: 33 patients spanning the full spectrum of NAFLD from the previously described Amsterdam NAFLD-NASH cohort (ANCHOR) underwent a multiparametric MRI of both liver and pancreas, in addition to a liver biopsy. Biopsies were analyzed in tandem by two independent liver pathologists using the NASH-CRN scoring system and EPOS fibrosis scale. MRI evaluation of the pancreas consisted of PDFF sequences, and of T1 mapping obtained using modified Look Locker inversion recovery (MOLLI) acquisition sequences. PDFF values of three regions of interest (ROIs) in the pancreatic head, body and tail were used to establish an average fat percentage. Similarly, three ROIs were selected in different liver slices of the liver. The mean signal intensity was taken to calculate the PDFF using a multi-echo and multifrequency fat signal model to correct for T2* effects. Statistics were done using Spearman correlations and ANOVA tests in R.

Results: Surprisingly, pancreatic fat content as measured by MRI-PDFF did not correlate with MRI-PDFF of the liver. Moreover, MRI-PDFF of the pancreas did not correlate with hepatic steatosis, inflammation or fibrosis scores on histology. However, pancreatic T1 values were significantly correlated to hepatic portal inflammation as scored by the pathologists ($R = 0.58$, $p < 0.01$) and increased with higher portal inflammation scores ($p < 0.05$). Moreover, we detected a possible correlation between pancreatic PDFF and pancreatic T1 values ($R = 0.51$, $p = 0.052$).

Conclusion: Despite common dysmetabolic drivers, fat buildup in the pancreas seems to be a separate pathophysiological process from NAFLD, potentially reflecting a cellular difference: adipocyte infiltration in the pancreas versus lipid droplet accumulation in hepatocytes. However, increased T1 values of the pancreas, which are thought to reflect tissue inflammation and fibrosis, correlated with hepatic portal inflammation. This raises the hypothesis that pathophysiological processes in the pancreas may contribute to hepatic inflammation in NASH, potentially via the portal vein.

P06-04 Insulin resistance in obese children with non-alcoholic fatty liver fibrosis

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Background and aims: Insulin resistance markers are promising markers for non-invasive assessment of hepatocyte damage and metabolic risk in NAFLD children which may be useful for the selection of pediatric patients at risk of disease progression. The aim of the study was to investigate the association of insulin resistance markers with liver fibrosis presence in obese children with NAFLD.

Method: 40 obese children aged from 10 to 17 years (average age was 12.15 ± 2.51 years) were examined. Obesity was established by body mass index (BMI) calculation and comparison with the standard deviations of BMI values according to age and sex. The presence of liver fibrosis and steatosis was established by transient elastography (Fibroscan®502touch, Echosense, France). Children were divided into 4 groups according to transient elastography and BMI data: I group-13 children with NAFLD and liver fibrosis, II group-13 children with NAFLD without fibrosis, III group -14 obese children without NAFLD and fibrosis. The control group consisted of 10 children with normal weight without NAFLD and fibrosis. Serum insulin levels were determined by enzyme-linked immunoassay (DRG International, Inc., Germany). Assessment of insulin resistance was carried out with a homeostasis model with HOMA-IR calculation.

Results: A significant increase in median insulin level (3.1 times, $p < 0.05$) and HOMA-IR level (3.3 times, $p < 0.05$) in children of the I group was observed compared to the control group. Moreover, the median insulin and HOMA-IR levels in children of the I group also differed significantly from children of the II group (1.5 times, $p < 0.05$ and 1.6 times, $p < 0.05$, respectively) as well as from children of the III group (2.0 times, $p < 0.05$ and 2.4 times, $p < 0.05$, respectively). Children of the II group had 2.1 times higher median insulin and HOMA-IR levels ($p < 0.05$), and children of the III group had 1.5 times ($p < 0.05$) and 1.4 times ($p < 0.05$), respectively, higher levels compared to the control group. It was found a positive correlation between HOMA-IR and the liver fibrosis degree ($r = 0.373$; $p = 0.019$).

Conclusion: Liver fibrosis formation in NAFLD children is accompanied by a significant increase in insulin and HOMA-IR levels reflecting insulin resistance progression in these patients correlated with liver fibrosis grade.

P06-05 Prospective relationship between PCOS plus NAFLD at age 17 years, and insulin resistance and atherogenic dyslipidaemia 10 years later in a longitudinal cohort study

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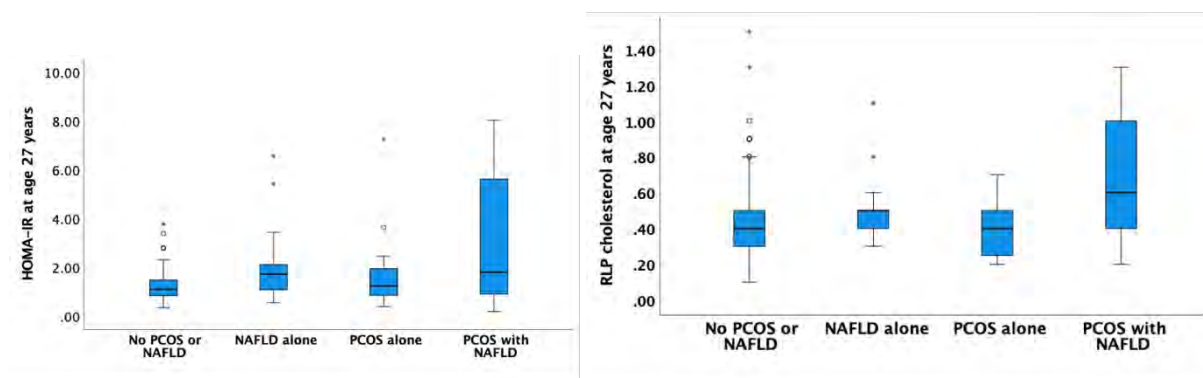
Background and aims: In women in their reproductive years non-alcoholic fatty liver disease (NAFLD) and polycystic ovary syndrome (PCOS) are the most common chronic liver and endocrine disorders respectively. NAFLD and PCOS are associated with increased cardiometabolic risk. We examined the prospective relationship between PCOS plus NAFLD in seventeen-year-old adolescents and insulin resistance and atherogenic dyslipidaemia 10 years later in a longitudinal cohort study.

Method: One hundred and ninety-nine community-based female adolescents participating in the Raine Study had assessments for PCOS and NAFLD, including anthropometry, blood biochemistry, pelvic and abdominal ultrasound. A diagnosis of PCOS was made using Rotterdam criteria. NAFLD was diagnosed based on liver echotexture, deep attenuation, and vessel blurring characteristics. Ten years later, during the 27-year follow-up, assessments including anthropometry and fasting blood tests were performed. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and remnant lipoprotein cholesterol (RLP-C) were calculated. Associations were sought between NAFLD with or without PCOS, PCOS alone and neither PCOS nor NAFLD, and HOMA-IR and RLP-C.

Results: At age 17 years, 37/199 (18.6%) had NAFLD, 20/199 (10.1%) had PCOS without NAFLD and 142 (71.3%) had neither NAFLD nor PCOS. Amongst those with NAFLD, 12/37 (32.4%) also had PCOS. Those with NAFLD combined with PCOS had a higher waist circumference, body mass index, subcutaneous fat, serum high sensitivity CRP, free and total testosterone, ferritin, but lower SHBG compared with those with NAFLD alone, PCOS alone or neither ($p < 0.05$ for all). Subsequently, ten years later when aged 27 years, women previously diagnosed with NAFLD combined with PCOS had higher mean [SD] fasting serum glucose (5.1 [1.6] vs. 4.6 [0.4] mmol/L, $p = 0.009$). Adolescents with PCOS combined with NAFLD were more insulin resistant and had higher RLP-C than other adolescents during adulthood (Figure 1), though there was no significant difference in serum glucose or liver transaminases.

Conclusion: NAFLD combined with PCOS in adolescents is associated with insulin resistance and atherogenic dyslipidaemia during adulthood ten years later.

Figure: 1. Association between HOMA-IR and RLP-C and NAFLD and/or PCOS 10 years earlier.



P06-06 Presumed NASH fibrosis as per non-invasive screening blood marker LIVERFAST-GP+ is predictive for Covid-19 short-term severe outcome

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Background and aims: Patients with COVID-19 caused by the SARS-CoV-2 virus human infection are at higher risk for severe outcome (SO) due to older age and underlying medical conditions, including chronic liver disease (CLD). Subjects with NAFLD/NASH may suffer from metabolic comorbidities such as diabetes, hypertension and obesity, putting them at increased risk of severe COVID-19. Data collected from worldwide multicenter registries as COVID-Hep (<https://covid-hep.net>) and SECURE-Cirrhosis (<https://covidcirrhosis.web.unc.edu/>) in patient with pre-existing CLD, suggested an incremental increased risk of ICU and death with each liver fibrosis stage along with patient's age. LIVERFAST-GP+ is an AI developed algorithm that utilizes a combination of serum biomarkers (liver enzymes, lipid panel, fasting glucose and total bilirubin) and anthropometrics that is intended to aid in the screening of NAFLD and its clinical category staging. Aimed to demonstrate that LIVERFAST-GP+ for liver fibrosis risk category provides prognostication for the short-term risk of developing COVID-19 SO.

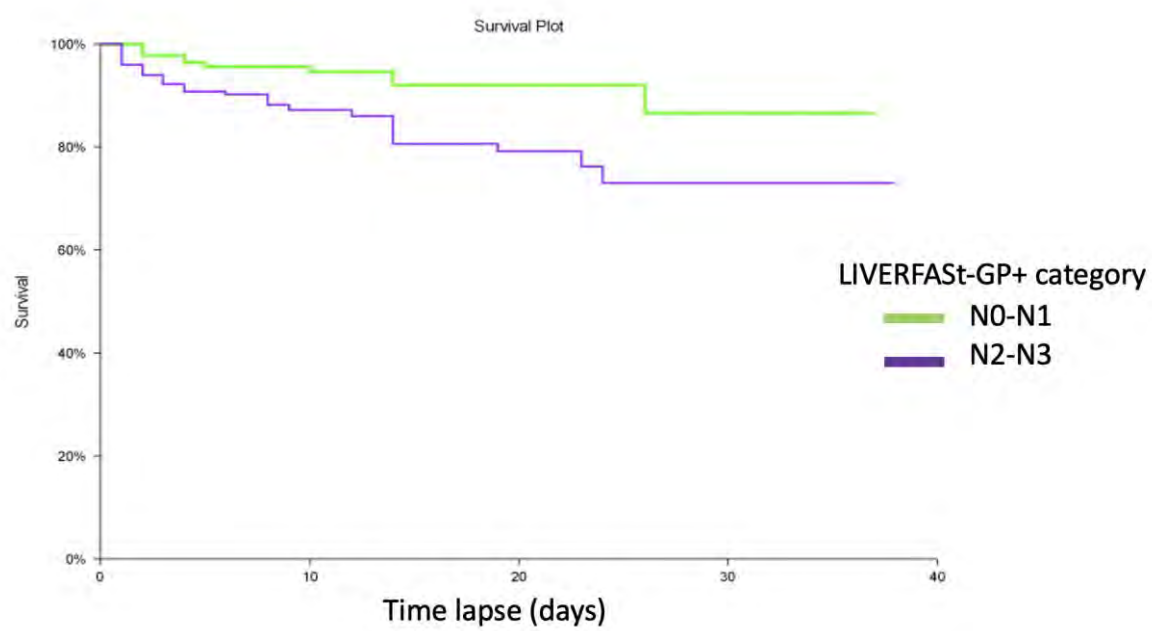
Method: N = 366 adult patients diagnosed with COVID-19 were included from the first wave from early stage asymptomatic through non-malignant late severe stage of the disease in one center of the EU Cohort of International Severe Acute Respiratory Infection Consortium (ISARIC).

SO were defined based on the need of invasive oxygen therapy (tracheal intubation), prone ventilation, ECMO, renal replacement therapy (RRT), ICU transfer inside the hospital, need of inotrope or dopamine therapy and cardiac arrest and palliative discharge.

LIVERFAST-GP+ is a screening tool providing a semiquantitative result in four categories N0 no presumed fibrosis/steatosis; N1 presumed steatosis only; N2 presumed mild/moderate fibrosis; N3 presumed bridging fibrosis (F3F4 stages).

Results: N = 33 patients had severe outcomes. Median (range) follow-up time until outcome 14 days (2-38 days). Patients with presumed liver fibrosis as per LIVERFAST-GP+ (N2-N3 category) had a lower cumulative survival (SE) during hospitalization without developing SO compared with patients without presumed fibrosis or normal liver (N0-N1): 93.91% (0.01) vs 94.18% (0.02), respectively, logrank $p < 0.01$. (Figure 1) Higher observed events rate 40/230 (17.4%) versus 10/136 (7.4%) for SO in N2-N3 group vs N0-N1, respectively with Cox Mantel HR (95%CI) 2.58 (1.46-4.55) versus 0.30 (0.22-0.68), $p < 0.01$.

Conclusion: LIVERFAST-GP+ category indicating the presence of liver fibrosis and of bridging fibrosis, respectively provides an accurate prediction of the risk to develop SO or death.

Figure:

P06-07 Novel MAFLD classification: a more realistic concept of fatty liver disease

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Background and aims: A terminology-change from non-alcoholic fatty liver disease (NAFLD) to metabolic associated fatty liver disease (MAFLD) has recently been proposed. MAFLD is a novel concept based on “positive criteria” that accurately reflects metabolic dysfunction, regardless of alcohol consumption or concomitant diseases. We aim to evaluate the importance of the novel MAFLD classification, by reclassifying our study cohort according to MAFLD and NAFLD definition.

Method: This study was conducted at the outpatient Hepatology Clinic of a tertiary University hospital, where patients were followed due to fatty liver disease by a multidisciplinary team.

Results: Amongst overall population (n = 206), there were 121 males (58.7%), with a mean age of 54 ± 14.3 years at diagnosis. Mean body mass index (BMI) was 32.49 ± 6.60 Kg/m². 146 patients met diagnostic criteria for NAFLD (70.9%) while 198 (96.1%) fulfil MAFLD criteria. Only 8/206 were NAFLD, non-MAFLD patients. Analyzing MAFLD non-NAFLD patients (n = 60), 76.5% had excessive alcohol consumption (≥20g/per day in women and ≥30g in men), and 14/60 (23.3%) had another etiology for liver disease. We further analyze MAFLD non-NAFLD patients (n = 60), according to metabolic risk factors and we did not find any significant difference in prevalence of arterial hypertension (p = 0.224), dyslipidemia (p = 0.998), diabetes mellitus (p = 0.678) and overweight (BMI ≥ 25 kg/m²) (p = 0.355). From our total cohort, 29.0% had advanced fibrosis (Fibroscan ≥ 10.4 kPa), from which 57.8% were MAFLD non-NAFLD patients. The median FIB-4 score index was 1.09.

Non-NAFLD group more frequently had advanced fibrosis (31.7 v 18.2%, p = 0.035) and showed higher values of FIB-4 score than non NAFLD group (median 1.25 VS 1.01, p = 0.012). Evaluating the degree of severe hepatic steatosis, with controlled attenuation parameter (CAP > 320 dB/m), no statistical difference between the groups was found (p = 0.435).

Conclusion: Patients with fatty liver disease frequently fulfill MAFLD criteria, with is a substantial overlap between NAFLD and MAFLD. A significant proportion of MAFLD patients not fulfilling NAFLD criteria, had advanced fibrosis, what is probably due to excessive alcohol consumption. Our results are in agreement with previous similar studies, emphasizing the importance of a more umbrella concept, such as MAFLD or FLD (Fatty Liver Disease) where the contribution of excessive alcohol consumption or associated liver diseases can be better appreciated.

P06-12 The use of spleen stiffness measurement as non-invasive tool to identify histological cirrhosis in individuals with non-alcoholic fatty liver disease

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Background and aims: Individuals with Non-Alcoholic Fatty Liver Disease (NAFLD) may have impaired liver-spleen axis due to the chronic, low-grade splanchnic inflammation, leading to spleen alterations even in the absence of portal hypertension. We aimed to explore the accuracy of spleen stiffness measurement (SSM) as a non-invasive tool to detect advanced fibrosis in individuals with biopsy-proven NAFLD.

Method: We retrospectively included patients with biopsy-proven NAFLD of any stage of fibrosis and available SSM. No patients had clinical signs of cirrhosis. Histological fibrosis was staged using the Metavir score (stages 0-4). Clinical and biochemical data were collected at the time of the biopsy. Liver stiffness measurement (LSM) and SSM were performed within one month from diagnosis by transient elastography using Fibroscan F630 using dedicated probes (50 Hz for the liver and 100 Hz for the spleen).

Results: A total of 40 patients were included, of which 15 had fibrosis stages 0-3 (F0-3) and 25 had histological cirrhosis (F4). Median age was 63.5 [IQR 53.0-68.5] years and 60% were male. Median Body Mass Index (BMI) was 31.7 [IQR 26.7-35.6] kg/m² and type 2 diabetes was present in 52.5% of cases. Median longitudinal spleen size was not different between F4 and F0-3 patients (14.7 [IQR 12.1-16.4] cm, $p = 0.133$) and was correlated with SSM values ($r = 0.30$, $p = 0.024$). Median SSM was 45.9 [IQR 27.9-73.1] kPa, with higher values in the F4 group, compared to F0-3 patients: 64.2 [IQR 33.9-74.1] kPa versus 26.3 [IQR 16.2-37.0] kPa, $p = 0.008$. LSM had a median value of 17.7 [IQR 10.4-28.3] kPa and was correlated with SSM ($r = 0.43$, $p = 0.031$). SSM could discriminate between F0-3 and F4 after adjusting for age, gender and BMI (aOR 1.04 [95% CI 1.01-1.09], $p = 0.045$), with a cut-off of 37.0 kPa by Youden Index (Se 72%, Sp 79%, PPV 85.7%, NPV 61.1%) and an Area Under the Curve (AUC) of 0.76. In this cohort, LSM identified histological F4 with an AUC of 0.780 at a cut-off of 9.7 kPa (Se 95%, Sp 65%, PPV 87.2%, NPV 81.8%). At the Delong test for AUC comparisons, no difference was detected between LSM and SSM ($p = 0.857$).

Conclusion: SSM is an accurate and additional marker of cirrhosis in patients with NAFLD. The correlation with LSM and the ability to detect advanced fibrosis using a threshold of 37 kPa suggests that SSM adds additional and valuable information on the effects of liver fibrosis even in asymptomatic patients.

Boehringer-Ingelheim sponsored the study.

P06-13-YI Free light chains: a new potential biomarker for disease stratification in non-alcoholic steatohepatitis

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Background and aims: NAFLD is the most common chronic liver disease worldwide. Almost 20% of NAFLD patients develop steatohepatitis (NASH) characterized by hepatocyte ballooning, lobular inflammation and progressive fibrosis. Overexpression of pro-inflammatory biomarkers (IL1-beta, IL6, TNF-alfa) is a key feature of the inflammation process of NASH.

Polyclonal free light chains (FLC) reflect B cell activation and could give insight into the activity of the adaptive immune system in a variety of inflammatory conditions. The aim of this study is to evaluate the potential role of FLC as biomarker of inflammation and fibrosis in NAFLD/NASH.

Method: We enrolled 187 patients with metabolic liver disease at Liver Outpatient clinic at Policlinico A. Gemelli: 48 with NAFLD, 85 with NASH and 54 with compensated liver cirrhosis. Diagnosis of NASH was histologically assessed. Medical and pharmacological anamnesis, anthropometric measurements and laboratory tests (FLC included, λ and κ) were obtained for all patients.

Results: Total FLC ($\lambda + \kappa$) were significantly higher in patients with cirrhosis than in patients with NAFLD or NASH (125.2 vs 33.3 mg/L $p < 0.01$). Total FLC, λ and κ were higher in NASH patients than in NAFLD although non significantly (respectively 32.4 vs 34.5 mg/L $p = 0.2$, 13.2 vs 15.0 mg/L $p = 0.2$, 19.1 vs 19.9 mg/L $p = 0.8$). In patients with NASH, total FLC are higher in patients with advanced fibrosis ($F > 2$, 39.0 vs 30.3, $p = 0.03$). Total FLC are associated to advanced fibrosis independently from age, sex, BMI and diabetes (OR 1.04, CI 1.00-1.08, $p = 0.04$).

Conclusion: This study showed that FLC concentration is significantly higher in patients with cirrhosis than in patients with NAFLD/NASH. In NASH patients, advanced fibrosis is associated with a higher serum concentration of total FLC. Serum FLC may represent a useful tool in grading and staging metabolic liver disease.

P06-16 The controlled attenuation parameter has limited value in assessing the degree of steatosis in different stages of compensated chronic liver disease

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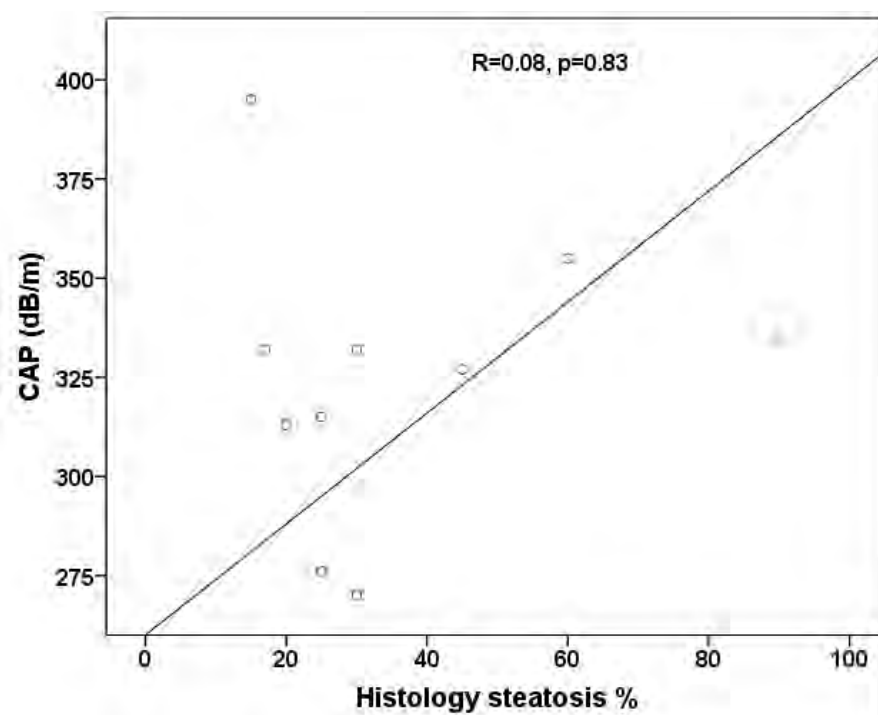
Background and aims: Liver biopsy is the gold standard method to assess liver injury, including steatosis, necroinflammation and fibrosis, but it is hampered by its invasive nature. For NAFLD, transient elastography (TE) can be used as a potential non-invasive substitute to liver biopsy. Continuous attenuation parameter (CAP) is a good diagnosis tool for hepatic steatosis, but its accuracy is dependent on body mass index (BMI), skin capsular distance and age. CAP estimates the percentage of hepatocytes affected by steatosis, while the histological examination can offer additional information related to the pathological changes in the parenchyma. The aim of this study was to assess the concordance between non-invasive methods for the quantification of steatosis parameters and histology in patients with compensated chronic liver disease.

Method: We screened the registry of our hospital for patients who had transjugular liver biopsy for etiological diagnosis of liver disease, during the COVID-19 pandemic (1 January 2020-8 March 2022). We excluded patients with decompensated cirrhosis or with a Child-Pugh score above 7 and with acute hepatitis. Hepatic TE quantifying liver stiffness (LSM) and CAP were measured using FibroScan® Expert 630 (v3.3.6). The liver biopsies were assessed by a single pathologist with experience in liver disease. Fibrosis was classified as absent (F0), mild (F1-2), severe (F3-4) on Fibroscan and histology. The statistical analysis was performed using SPSS® (v.22.0), considering $p < 0.05$ for statistical significance.

Results: From the 244 liver biopsies performed, 97 patients met the inclusion criteria, of whom 56 (57.7%) were male. The etiology for liver disease was alcohol in 17 (17.5%) patients, NASH in 14 (14.4%), viral in 19 (19.6%), autoimmune in 6 (6.2%), vascular disease in 19 (19.6%), cholestatic in 9 (13.4%) and other in 13 (13.4%). Cirrhosis was present at the moment of biopsy in 37 (38.1%) patients, with a median MELD-Na score of 9 (3). TE was performed in 77 (79.4%) patients, with a median LSM of 13 (19) and a mean CAP of 263 (± 58.5). The median % of Steatosis on histology was 10 (26). The CAP score did not correlate with the % of steatosis on the histology for patients with alcohol (Spearman's $\rho = 0.24$, $p = 0.41$) and NASH (Spearman's $\rho = 0.08$, $p = 0.83$) etiologies. The CAP value was significantly different between patients with mild fibrosis versus severe fibrosis on histology (246 ± 61.3 vs 277 ± 52.5 , $p = 0.02$). The fibrotic stages on TE matched those on biopsy for alcohol ($p = 0.001$), but not for NASH ($p = 0.13$) etiologies. There were no differences in LSM value in relation to the degree of steatosis (none, mild, moderate-severe), $p = 0.65$.

Conclusion: The CAP value is dependent on the etiology of liver disease, is influenced by liver fibrosis and it poorly correlates with the histology. For NASH patients, the CAP value should be carefully interpreted together with the LSM.

Figure:



P06-17 Cost-effectiveness of magnetic resonance elastography for non-alcoholic steatohepatitis fibrosis in the United Kingdom

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Background and aims: Magnetic Resonance Elastography (MRE) is a highly accurate non-invasive test (NIT) for identifying patients at low risk of fibrosis and who may not benefit from invasive testing in the form of liver biopsy. Our aim is appropriate to assess its cost-effectiveness in helping to avoid unnecessary liver biopsies in clinical management of non-alcoholic steatohepatitis (NASH) in the United Kingdom (U.K.)

Method: We compared the systemic cost of two approaches for stratification of patients with suspected NASH fibrosis in the U.K.. The current standard of care suggests liver biopsy for definitive staging if initial staging with non-invasive tests such as clinical/laboratory scoring are not conclusive. The other approach is identical to the standard of care approach except that MRE offered before biopsy to reduce the number of patients who are ultimately subjected to staging with biopsy.

The cost-efficacy model closely follows procedures used by the U.K. National Institute for Clinical Excellence (NICE). This approach evaluates the costs, quality-adjusted life-years (QALYs), and Incremental Cost Effectiveness Ratio (ICER) of two diagnostic pathways: "MRE before biopsy" and "liver biopsy only". The population of interest is patients who had indeterminate fibrosis assessment. The goal was to evaluate whether the "MRE before biopsy" pathway would remove the costs and lost QALYs arising from patients having unnecessary biopsies. For input data on the diagnostic performance of MRE, we used published meta-analyses in NASH populations. For input data on cost and QALYs for biopsy, we used published data from UK-centric studies.

Results: The model indicates that, for every thousand patients for whom initial fibrosis assessment is indeterminate, the "MRE before biopsy" pathway would avert a significant number of biopsies, resulting in substantial cost savings per 1000 patients and positive QALYs gained. The ICER is therefore negative, compared to a positive ICER of £20, 000-£30, 000 per QALY gained that is the current willingness to pay threshold in the U.K. As a result, this workflow would result in a net cost savings to the U.K. healthcare system over the current standard of care.

Conclusion: The incorporation of MRE would result in significant cost savings and thus be a highly cost-effective use of NHS resources.

Figure:

Fibrosis Stage	Biopsies Averted per 1000 patients using MRE before biopsy	Total Cost (or Savings) of Adding MRE per 1000 patients	QALYs Gained	ICER per QALY Gained (UK willingness to pay threshold: £20, 000-£30, 000)
≥F1	539-631	(£198, 640) to (£259, 713)	2.8 to 3.4	(£71, 408) to (£75, 769)
≥F2	673-705	(£293, 943) to (£312, 931)	4.4 to 4.4	(£67, 432) to (£71, 489)
≥F3	698-749	(£311, 383) to (£344, 936)	4.6 to 5.0	(£67, 602) to (£69, 453)
≥F4	792-829	(£375, 119) to (£399, 696)	5.4 to 5.7	(£68, 915) to (£69, 921)

P06-20 Predictive survival-time modelling of non-alcoholic steatohepatitis (NASH) fast progressors using real-world evidence

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Background and aims: Evidence suggests that a subset of patients with Non-Alcoholic Fatty Liver Disease (NAFLD) may progress faster to end stage liver disease via Non-alcoholic Steatohepatitis (NASH). There is a need for methods to identify such sub-populations of fast progressors in order to improve patient care and enable more effective clinical trials. Prior machine learning based approaches to identify progressors, e.g. using binary prediction of progression types, did not consider censored events due to death, transfer out of data source, or end of study period, which can reduce accuracy of the results. The aim of this study is to develop a machine learning *survival-time prediction* model that can identify NAFLD and NASH patients at risk of fast progression to end stage liver disease based on real-world data, taking censoring of events into account.

Method: We retrospectively evaluated patients in Optum® Clinformatics® Data Mart (de-identified US electronic health records, 2007-2021) aged ≥18 with the index-time being the first diagnosis of NAFLD or NASH based on ICD- codes with no prior liver disease. Progression time was defined as the time between index date and the first hard end point of NASH or censored at death, transfer out of data source, or end of study period.

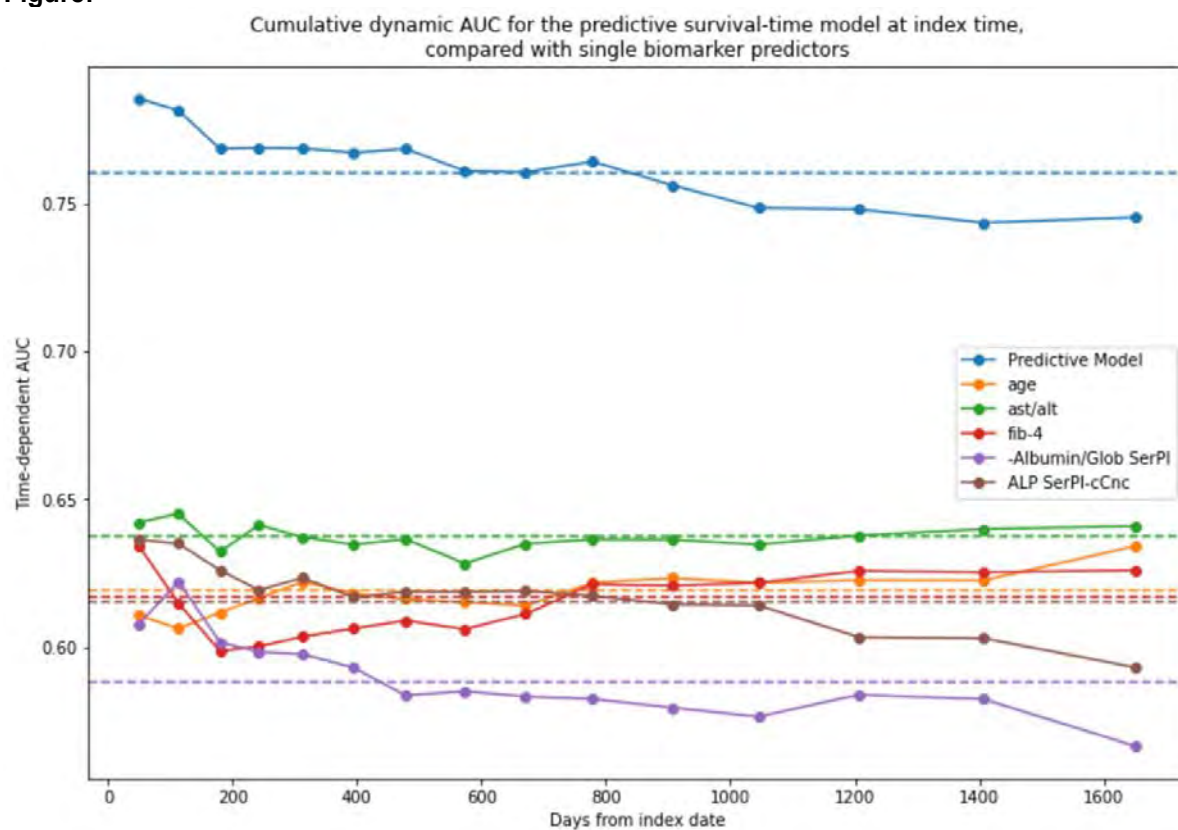
Progression times were predicted using a machine learning survival-time model robust to missing data, trained on sparse data and 80% of the subjects. Model performance was evaluated using the cumulative dynamic area-under-the-curve on the remaining subjects and compared with five single biomarker baseline models. The most important features were visualized using Shapley Additive exPlanations (SHAP).

Results: A final cohort of 260, 180 patients matched the inclusion and exclusion criteria, 3% of the patients had a severe end point observed, and the median progression time for these patients was 14 months.

The predictive model had an *average cumulative dynamic AUC of 0.76* for predicting progression times from index to the end of the study period, compared with 0.64 for the best performing baseline model, the AST/ALT ratio.

Conclusion: The predictive survival-time model outperforms individual biomarkers at characterizing NASH fast progressors both at index time and continuously until the time of event.

Future research of the model should include validation in other datasets, including patients in a range of geographic regions, of different ethnicities and with biopsy confirmed NASH.

Figure:

P06-21 Comparison of severity of liver damage, metabolic alterations and cardiovascular damage in patients with NAFLD attending the hepatology clinic over the last three decades

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Background and aims: Prevalence of non-alcoholic fatty liver disease (NAFLD) has been increasing over the last years paralleling the spread of metabolic disease. Aim: to assess whether severity of metabolic, liver and cardiovascular diseases changed over time in NAFLD patients.

Method: 413 patients (74% male, mean age 53±13 years) with NAFLD referring to the hepatology clinic between 1990 and 2021 were enrolled. All patients underwent a liver biopsy, excluded 37 subjects with a clinical diagnosis of cirrhosis. Metabolic parameters, early atherosclerosis (by carotid intima media thickness and plaques) and histological liver damage (by NAS score, presence of NASH and fibrosis) were assessed at the time of biopsy. The period of presentation was divided into 3 decades (1990-2000 n = 21, 2001-2011 n = 187, 2012-2021 n = 205). As for the small numbers of subjects in the first decade, we compared features of only between the last two ones.

Results: No differences in age of presentation and sex were found between decades. Conversely, obesity (45% vs 27%, $p < 0.001$), type 2 diabetes (T2DM) (36% vs 26%, $p = 0.03$) and hyperuricemia (51% vs 37%, $p = 0.006$) were more prevalent in the last decade compared to the second one, whereas hypertension and dyslipidemia remained stable over time. Liver damage was significantly more pronounced in the last decade compared to the previous (severe steatosis 38% vs 17%, $p < 0.001$, NASH 30% vs 11%, $p < 0.001$, fibrosis $\geq F2$ 36% vs 18%, $p = 0.02$, cirrhosis 19% vs 11%, $p = 0.02$). The presence of carotid plaques was also higher in the third decade compared to the second although it did not reach statistical significance (42% vs 32%, $p = 0.06$). Considering the whole cohort, T2DM was an independent risk factors for NASH (OR 2.1, 95% CI 1.7-4.0) and fibrosis $\geq F2$ (OR 3.2, 95% CI 1.7-6.0), whereas age (OR 1.04, 95% CI 1.01-1.07), obesity (OR 3.2, 95% CI 1.8-5.8) and presence of NASH (OR 5.4, 95% CI 2.8-10.6) were independently associated with fibrosis $\geq F2$. Finally, age (OR 1.1, 95% CI 1.07-1.2) and hypertension (OR 2.3, 95% CI 1.3-3.9) were independent risk factors for carotid plaques.

Conclusion: Over the past 10 years compared to previous decade, patients with NAFLD presented to observation with more severe liver disease, possibly paralleling the spread of diabetes and obesity. Our findings suggest the need, once a patient with NAFLD is diagnosed in primary care, to refer the patient to the hepatology center, promptly checking for hepatic fibrosis, especially if metabolic alterations coexist.

P06-22-YI The impact of metabolic comorbidities and alcohol consumption on FIB-4 and NFS performance in MAFLD: a multicentric preliminary data

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Background and aims: Metabolic dysfunction-associated fatty liver disease (MAFLD) is defined by the presence of liver steatosis and, at least, one metabolic comorbidity. Due to the invasiveness of liver biopsy, non-invasive tests (NITs) of fibrosis, such as Fibrosis-4 Index (FIB-4) and NAFLD fibrosis score (NFS) are widely used in NAFLD. FibroScan® accurately distinguishes advanced and earlier stages of fibrosis by liver stiffness measurement (LSM), with good concordance between LSM and the stage of fibrosis at liver biopsy. The term MAFLD and NAFLD are not interchangeable, and the impact of alcohol consumption is not well defined. We aimed to 1) compare the accuracy of NITs in comparison with LSM in MAFLD and 2) evaluate the impact of metabolic comorbidities and alcohol consumption on NITs performance.

Method: We enrolled 600 patients with MAFLD referred to liver clinic in Lisbon and Milan. We collected clinical and laboratory data and performed FibroScan® using M and XL probes according to the skin-liver capsule distance. LSM<7.9/≥10kPa and LSM<7.0/≥9.3 kPa for M/XL probes excludes and confirms advanced fibrosis). Daily alcohol consumption categories: abstainers, modest (<30 gr/20 gr), moderate (30-40 and 20-30 gr) and heavy (≥40 and ≥30 gr or binge drinking) for men and women respectively. FIB-4<1.45 and NFS<-1.455 ruled out advanced liver fibrosis while FIB-4>3.25 and NFS>0.676 suggested advanced fibrosis.

Results: Mean age was 54 yrs, 60% male, 54% were obese and 30% had diabetes. Regarding reported alcohol consumption, 61% were abstainers, 26% modest and 13% had moderate to high alcohol consumption. Seventy-three % had LSM<7.9 kPa, 15% LSM≥10 kPa, 74% had lower FIB-4, 4% higher and 22% indeterminate, 70% had lower NFS, 2% higher NFS and 28% indeterminate. Compared to LSM, FIB-4 and NFS had AUROCs of 0.58 and 0.52 for detecting advanced fibrosis and of 0.66 and 0.72 for the exclusion of significant fibrosis. Both FIB-4 and NFS didn't reach high sensitivity and specificity (≥80%). For both detection and exclusion of advanced liver fibrosis, FIB-4 performed worse in obese versus non-obese patients (AUROCs 0.55 vs 0.63; AUROCs 0.64 vs 0.72), while no differences were found regarding NFS (AUROCs 0.51 vs 0.52; AUROCs 0.71 vs 0.70). FIB-4 and NFS performed poorly in T2DM for advanced fibrosis (AUROCs <0.60), while no differences were noticed between diabetic and non-diabetic patients for the exclusion of significant fibrosis. There was a negative impact of alcohol consumption on diagnostic performance for advanced liver fibrosis, particularly for NFS (AUROCs 0.60 for moderate and 0.50 for high alcohol).

Conclusion: Metabolic comorbidities (diabetes and obesity) and alcohol consumption may influence the ability of the non-invasive fibrosis score in identifying advanced liver fibrosis in MAFLD. Revision of values may be necessary to avoid misdiagnosis in patients with advanced liver disease.

P06-23 Prevalence of non-alcoholic fatty liver disease (NAFLD) in middle-aged men and women with overweight and normal liver enzymes, and diagnostic accuracy of non-invasive proxies

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Background and aims: The prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing at an alarming rate. Elevated liver enzymes are a primal reason to refer patients for further testing. However, normal liver enzymes may not exclude the presence of NAFLD. Therefore, we examined the prevalence of NAFLD in a middle-aged population with overweight and normal liver enzymes. In addition, we examined the accuracy of five sets of non-invasive proxies for NAFLD.

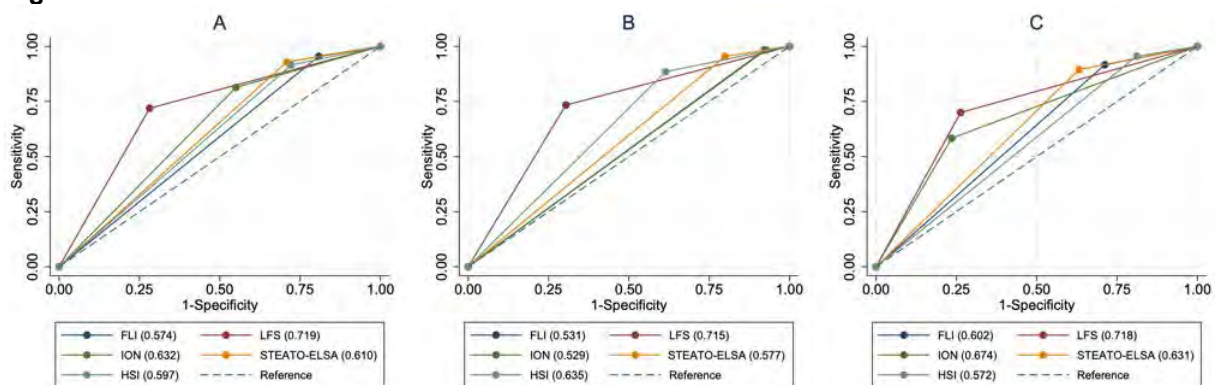
Method: We included 1, 038 participants from the Netherlands Epidemiology of Obesity (NEO) study with BMI ≥ 25 kg/m² and liver enzymes (AST, ALT, gGT) within the normal range. To assess the diagnostic accuracy of biomarkers scores (Fatty Liver Index (FLI), Liver Fat Score (LFS), Index of NASH (ION), STEATO-ELSA, and Hepatic Steatosis Index (HSI)), the study population was subdivided in individuals with and without NAFLD, defined as hepatic triglyceride content (HTGC) $\geq 5.56\%$ as measured by Proton Magnetic Resonance Spectroscopy (¹H-MRS).

Results: Participants (mean age 56 years, 49% women), had a median BMI of 29.6 kg/m² and a median HTGC of 4.4%. NAFLD was present in 42% of participants and was more common in men than in women, with respectively 47% and 36% being affected. The LFS had both a sensitivity and specificity of 0.72, and an

Area Under the Curve (AUC) of 0.72. The other sets of biomarkers had lower diagnostic accuracies.

Conclusion: The prevalence of NAFLD in middle-aged men and women with overweight and normal liver enzymes is over 40%. We identified the LFS as a useful proxy to identify this high-risk population.

Figure:



ROC-curves of the five biomarker scores for the entire studied population (A), men (B) and women (C).

P06-25 Patients with compensated cirrhosis due to NASH show decreased plasma levels of sphingolipids and MCP-1 and increased leptin

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Background and aims: Increased plasma ceramide concentrations have been associated to non-alcoholic fatty liver disease (NAFLD), increased risk of diabetes and cardiovascular events. However, in patients with liver cirrhosis (mainly due to viral infection or excess alcohol intake) plasma concentrations of ceramides (CER) and sphingomyelins (SM) are often found decreased. Thus, we wanted to explore if patients with compensated cirrhosis due to NASH have altered plasma concentrations of sphingolipids and their relationship with insulin resistance (IR).

Method: We studied 109 subjects with NAFLD (age 51, BMI 28.4 ± 0.5 kg/m², 66% males), 28 with compensated cirrhosis (G3), 40 with active NASH and significant fibrosis (G2: NASH + NAS ≥ 4 + F ≥ 2) and 41 with less severe NAFL/NASH (G1). We measured sphingolipids (CER and SM) by LC-MS QTOF in plasma samples, together with markers of inflammation (MCP-1 and leptin) and IR (HOMA-IR).

Results: Patients with cirrhosis (G3) were slightly older than G1 and G2 but with similar BMI and HOMA-IR. There was a stepwise increase in leptin concentrations from G1 to G3 (3.9 ± 0.6 vs 7.3 ± 1.0 vs 9.0 ± 1.8 ng/ml, $p < 0.009$) and a decrease in MCP-1 (133 ± 10 vs 104 ± 11 vs 99 ± 16 pg/ml, $p < 0.02$). CER and SM were similar in G1 and G2 but reduced by half in G3 ($p < 0.001$). sphingolipids were positively correlated with MCP-1 ($r > 0.21$, $p < 0.005$) but not with leptin concentrations or HOMA-IR.

Conclusion: Analysis of lipids in blood from patients with compensated cirrhosis due to NASH reveals an increase in leptin concentrations (regardless of BMI) and a general suppression of sphingolipid levels compared to patients with active NASH and F ≥ 2 .

P07-01 Multicenter validation of FIB-6 as a novel machine learning non-invasive score to rule out liver cirrhosis in biopsy proven MAFLD

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Background and aims: Non-invasive tests (NITs) are urgently required to evaluate hepatic fibrosis in MAFLD. We previously developed and validated a non-invasive diagnostic index based on routine laboratory parameters for predicting the stage of hepatic fibrosis in patients with chronic hepatitis C (HCV) called FIB-6 through machine learning with random forests algorithm using retrospective data of 7238 biopsy proven chronic hepatitis C (CHC) patients. Our aim is to validate this novel score in patients with MAFLD.

Method: Performance of the new score was externally validated in cohorts from one site in Egypt (n = 674) and in 5 different countries (n = 1798) in Iran, KSA, Greece, Turkey and Oman. Biopsy samples were scored by experienced pathologists who used the METAVIR scoring system. Results were also compared with three established tools (FIB-4, APRI, and AAR).

Results: 2472 patients met the criteria, and their liver biopsy results were included for analysis. Using the optimal cutoffs in the data of FIB-6 indicated a reliable performance in diagnosing cirrhosis, severe fibrosis, and significant fibrosis. Results indicated good sensitivity and NPV. Sensitivity = 70.5%, specificity = 62.9%. PPV = 15.0% and NPV = 95.8% for diagnosis of cirrhosis. For diagnosis of severe fibrosis (F3 and F4), the results were 86.5%, 24.0%, 15.1% and 91.9% respectively, while for diagnosis of significant fibrosis (F2, F3 and F4), the results were 87.0%, 16.4%, 24.8% and 80.0%. Comparing of the results of FIB-6 rule-out cutoffs with those of FIB-4, APRI, and AAR showed that in ruling out severe fibrosis and cirrhosis, FIB-6 gave the highest sensitivity and NPV (97.0% and 94.7%), as compared to FIB-4 (71.6% and 94.7%), APRI (36.4% and 90.7%), and AAR (61.2% and 90.9%).

Conclusion: FIB-6 score is an accurate, simple, NIT for ruling out advanced fibrosis and liver cirrhosis in patients with MAFLD better than APRI, FIB 4 and AAR.

P07-02 Metabolic syndrome correlates to a higher risk of fibrosis by surrogate scores compared to patients who do not fully meet the syndrome diagnostic criteria

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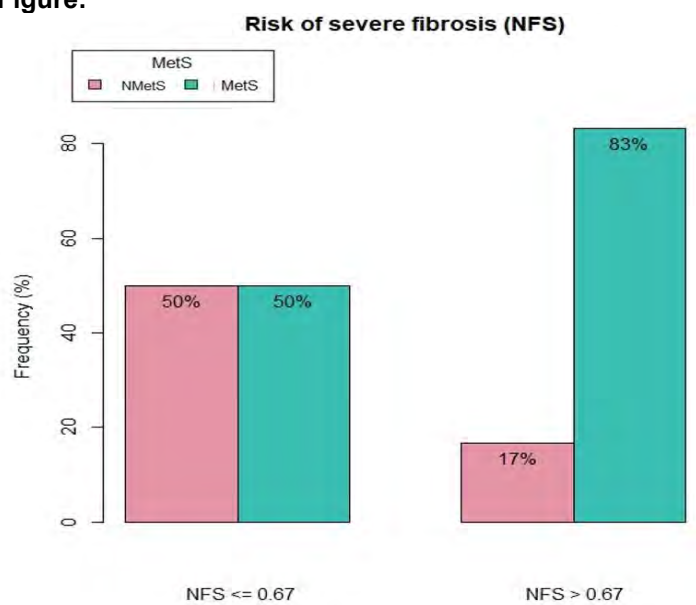
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Background and aims: Metabolic Syndrome (MetS) has been growing in prevalence for the past decades. Non-Alcoholic Fatty Liver Disease (NAFLD) and Non-Alcoholic Steato-Hepatitis (NASH) are the hepatic manifestations of MetS and are becoming the most prevalent cause of end-stage liver disease in the western world. In the context of our department's free-access screening for NASH in the Emilia-Romagna population, we evaluated the differences in FIB-4, NFS indexes, elastography's LSM and CAP among subjects with MetS and without it (NMetS).

Method: As of June 30th 2022, out of the 134 screened patients we selected a cohort of subjects with both indirect fibrosis indexes and LSM and CAP (measured by FibroScanTM). Age, BMI, caloric intake (CI) and Basal Metabolic Rate (BMR) were also collected for each patient in the study. NCEP criteria were used to diagnose MetS, which is, at least 3 of the following simultaneous findings: fasting plasma glucose ≥ 100 mg/dl; serum triglycerides ≥ 150 mg/dl (or treatment); serum HDL cholesterol < 40 mg/dl (< 50 mg/dl if females); systolic blood pressure $\geq 130/85$ mmHg (or treatment); waist circumference > 102 cm (> 88 cm in females). The cohort was then grouped in MetS and NMetS. According to the normality of distributions, we tested the null hypothesis of equivalence with a 99% CI of the values (reported as median \pm IQR) using either one-way ANOVA, Welch test or Kruskal-Wallis test; proportion tests were evaluated with a Pearson's Chi-squared test.

Results: The cohort (n = 106) is composed of 55 MetS and 51 NMetS, of which 65 females and 41 males equally distributed, of an age ranging from 18 up to 81; 8 subjects had normal weight, 33 were overweight, 65 had obesity (46 class I, 12 class II, 7 class III). Compared to the NMetS, the MetS group displayed statistically significant (p < 0.01) higher values of FIB-4 (0.99 ± 0.73 vs 0.75 ± 0.85), NFS (-0.35 ± 2.56 vs -1.43 ± 1.92), LSM (5.1 ± 3.2 vs 4.2 ± 1.3) and CAP (298 ± 65 vs 244 ± 57). Weight categories distribution, CI and BMR didn't show statistically significant differences between the two groups (p > 0.05). Fibrosis subgroups by FIB-4 values were equally represented in the groups without significant difference (p > 0.05).

Conclusion: While screening for NASH, MetS patients displayed higher FIB-4, NFS, LSM and CAP values compared to subjects who did not meet the diagnostic criteria for MetS. In particular, MetS patients showed: a 1.9x higher risk (81% vs 43%) of having liver steatosis as showed by CAP > 248 dB/m (p < 0.001); a 4x higher risk (16% vs 4%) of having an intermediate risk of liver fibrosis as showed by LSM > 8 kPa compatible with (p < 0.05); and a 3.9x higher risk (31% vs 8%) of having severe fibrosis as suggested by NFS > 0.67 (p < 0.01) (figure). These findings show a clear correlation between MetS and liver fibrosis, therefore, an appropriate management of the syndrome can be assumed to help to prevent the disease progression towards fibrotic-NASH.

Figure:

P07-03-YI The effects of a structured dietetic intervention in patients with NAFLD

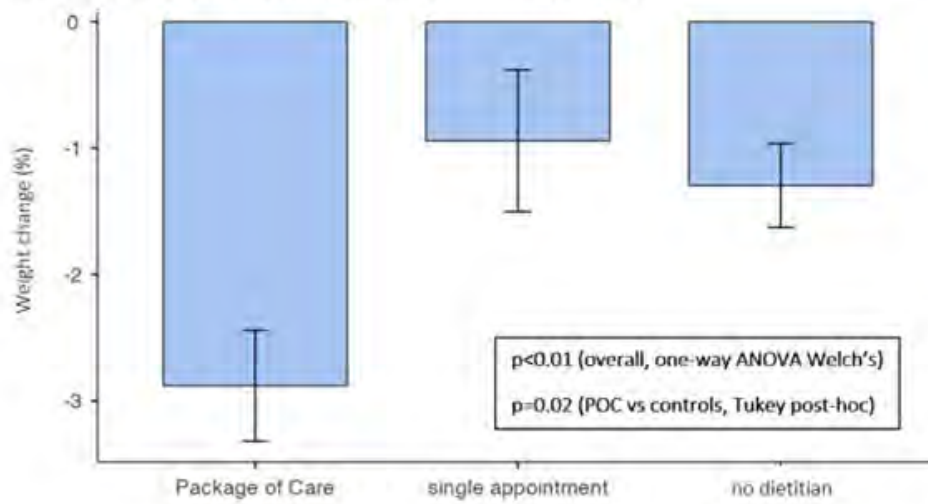
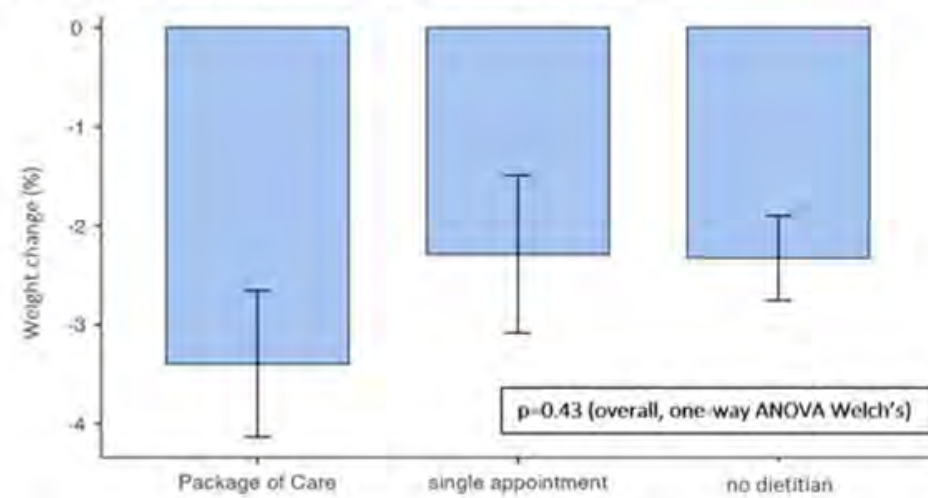
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Background and aims: Diet and lifestyle modification to aid weight loss remains the cornerstone of NAFLD management. Since 2014, selected patients attending a multi-disciplinary NAFLD clinic have been referred to a specialist dietitian. They either receive a single appointment (SA) with follow-up after 3 months and as required thereafter, or a comprehensive, structured package of care (POC) consisting of 4 sessions within 6 months. Our aim was to assess whether this dietetic POC is associated with more favourable outcomes.

Method: In this retrospective service evaluation, we reviewed the outcomes of patients in 3 groups: those who had completed $\geq 3/4$ sessions in the dietetic POC, those who had completed a SA, and those who had not seen a specialist dietitian (controls). In the POC and SA groups, weight was recorded from the first dietetic appointment (baseline), final dietetic appointment (intervention end), and at 18 months from baseline (long term). For controls, weight at baseline, 6 months, and 18 months was recorded. Fibroscan (TE) results before and after intervention were recorded. Significant improvement in TE was considered $\geq 20\%$ reduction if baseline $\geq 6\text{kPa}$; significant worsening was considered $\geq 20\%$ increase if baseline $\geq 5\text{kPa}$, or a change to $\geq 6\text{kPa}$ if baseline $< 5\text{kPa}$.

Results: 381 patients were included: 89 POC, 49 SA, and 243 controls. Mean weight changes from baseline to intervention end were -2.9kg (-2.8%) for POC, -1kg (-0.9%) for SA, and -1.3kg (-1.2%) for controls (Figure 1a). Patients who received the POC lost significantly more weight (both kg and %) than controls (Tukey post-hoc test, $p = 0.02$). There was also a significant difference between POC and controls in weight change of $\geq 5\%$ (X^2 , $p = 0.017$) but not $\geq 7\%$ or $\geq 10\%$. There was no significant difference in weight loss between POC and SA, or SA and controls. At multivariate logistic analysis, the POC was associated with $\geq 5\%$ weight loss (OR 2.15, $p < 0.01$) independent of time interval, age, sex and metabolic comorbidities. At long term (mean 18 months) follow-up, all groups achieved further weight loss (-3.4% SOC, -2.3% SA, -2.3% controls, Figure 1b) but there was no longer a significant difference in weight change from baseline between groups. Regarding liver stiffness, the mean interval between Fibroscans was 24 months. Overall, 28.1% patients had a significant improvement and 22.7% had a significant worsening. There was no significant difference in changes of TE between groups. Overall, $\geq 5\%$ weight loss after 6 or 18 months was associated with a significant improvement in TE (X^2 , $p < 0.01$).

Conclusion: A structured dietetics POC consisting of 3 or 4 sessions over 6 months was associated with greater rate of $\geq 5\%$ weight loss than controls, and the effect was maintained beyond the end of the intervention. $\geq 5\%$ weight loss after 6 or 18 months was associated with significant improvement in liver stiffness.

Figure:**1a. Mean weight change (%) from baseline to end of intervention (6 months)****1b. Mean weight change (%) from baseline to long term follow up (18 months)**

P07-04 NASH PASS®: a registry to inform study design and accelerate patient enrollment

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Background and aims: Despite the reported high prevalence of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) in the US, trials have challenges with slow enrolment. The aim of the NASH PASS (NASH PATIENT ACCESS AND SCREENING STRATEGIES) registry is to provide a platform to model study design criteria in a community-based population to favourably accelerate clinical development programs. ProSciento's experience applying this model has resulted in expedited study start-up of interventional clinical trials by more than 1 month on average, reduced screen failures by 15-30%, and increased enrolment rate to more than double compared to industry average.

Method: NASH PASS is a cross-sectional diagnostic study and registry-based IRB approved clinical research protocol with detailed prospective data collection on subsets of participants. Individuals at high risk for developing NAFLD or NASH are invited to undergo comprehensive screening to determine disease status. Participants can be followed for up to 10 years. NASH PASS data was utilized to analyse baseline characteristics of individuals at high risk for developing NAFLD. Sample t-tests were performed to compare differences among Type 2 diabetic (T2DM) and non-diabetic participants.

Results: Overall, 4041 participants were included between April 2019 and June 2022. The mean (SD) age is 55.5 (12.3) years, BMI is 34.9 (6.1) kg/m², 53.6% are females, 52% are Hispanic, and 56% have T2DM. 33% (n = 1335) of participants met criteria for NAFLD diagnosis. 50% have a diagnosis of T2DM, 52% are females and 50% are Hispanics. Their mean CAP score is 341.6 (29.1) dB/m, AST is 19.53 (8.3) U/L, and ALT is 25.7 (15.3) IU/L.

The NAFLD cohort characteristics are broken down by history of T2DM and summarized in Table 1. There are statistically significant differences in NAFLD specific parameters between non-diabetic and T2DM participants.

Conclusion: The NASH PASS registry is a research database from a growing and well-characterized population that is a powerful tool to analyse and inform study design development, facilitating enrolment for NAFLD clinical trials. The result of this analysis helps better understand this population, increasing access to it.

It also provides a pathway for further research and educational opportunities in earlier identification of the NAFLD patient population.

Figure:

Table 1. Differences between Non-diabetic and T2DM participants						
	Non-diabetes (n = 671)		T2DM (n = 664)		t	p value
	Mean	SD	Mean	SD		
CAP score	339.8	28.3	343.3	30.7	-1.86	0.07
AST	20.9	8.5	17.8	6.5	5.34	<0.001
ALT	28.2	17.7	23.2	11.9	4.61	<0.001
Insulin	17.7	12.0	21.3	20.8	-2.57	0.010
HOMA-IR	4.5	3.8	8.3	10.6	-5.61	<0.001
Glucose	100.6	23.0	151.4	67.3	-12.05	<0.001

*Equal variances not assumed

P07-05 Triglycerides and glucose (TyG) index but not homeostasis model assessment (HOMA) index predicts cardiovascular events in patients with non-alcoholic fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease is the most common liver disease worldwide and its prevalence steadily grow. The spread of NAFLD in Western countries is strongly associated with the increasing prevalence of obesity and T2DM due to the changing lifestyle and dietary habits. Some authors consider NAFLD as the hepatic manifestation of fatty liver. Aim of the study was to investigate which of TyG and HOMA-IR indexes better predict cardiovascular events in NAFLD patients.

Method: the present study is a post-hoc analysis of Plinio Study (Progression of Liver Damage and Cardiometabolic Disorders in Non-alcoholic Fatty Liver Disease: an Observational Cohort Study. ClinicalTrials.gov Identifier: NCT04036357). Plinio study includes dysmetabolic patients investigated for the presence of NAFLD. TyG index (Tryglicerides and glucose index- $\ln[\text{Fasting triglyceride (mg/dl)} \times \text{Fasting glucose (mg/dl)}]/2$) and HOMA-IR (Homeostatic Model Assessment for Insulin Resistance- $\text{Fasting insulin (mg/dl)} \times \text{Fasting glucose (mg/dl)}/405$) were calculated as insulin resistance scores. NAFLD fibrosis score (NFS) was calculated as non-invasive markers of fibrosis. Data on Major Adverse Cardiovascular Events (MACEs) were collected during the follow-up.

Results: Plinio study included 1039 patients, 826 with NAFLD (79.5%) and 213 without NAFLD (20.5%). Patients with NAFLD had higher median TyG Index (4.8 ± 0.3 vs 4.6 ± 0.2 , $p < 0.001$) and HOMA-IR (4.1 ± 3.4 vs 1.6 ± 1.7 , $p < 0.001$). Among patients with NAFLD, those with positive NFS ($n = 34$) had higher median TyG (4.9 ± 0.3 vs 4.7 ± 0.3 , $p < 0.001$) and median HOMA-IR (6.0 ± 4.4 vs 3.6 ± 2.4 , $p < 0.01$) in comparison to those with negative NFS ($n = 398$). Patients with NAFLD were followed-up for a median of 43 [21-70] months yielding 3307 person-years of observation. During the follow-up were observed 57 MACEs. HOMA-IR didn't predict MACEs, while TyG index predicted MACEs (III tertile vs. I tertile HR: 2.14, $p < 0.05$) after adjustment for age, sex, obesity and diabetes.

Conclusion: unlike the HOMA-IR, TyG index predicts MACEs in NAFLD patients, and its use could help identify patients in need of more careful cardiovascular prevention.

P07-08 Quantification of hepatic steatosis with a novel attenuation imaging ultrasound technique (QAI): preliminary findings on reproducibility and diagnostic accuracy

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Background and aims: In recent years various ultrasound (US) techniques, such as attenuation imaging, have been developed to quantitatively assess the hepatic fat content. The aim of the study was to assess technical feasibility and reproducibility (both intra- and inter-observer) of attenuation imaging with QAI (Esaote) in healthy volunteers and in patients with suspected steatosis. The secondary aim was to evaluate the correlation of QAI with hepatic steatosis and to assess diagnostic accuracy for steatosis quantification as compared to liver biopsy.

Method: This prospective study included two different study groups, composed of healthy volunteers (group 1, n = 25) and patients with suspected metabolic disease (group 2, n = 74) undergoing liver biopsy. In group 1 two operators performed both US and two sessions of attenuation imaging respectively. Intra-class correlation coefficients (ICCs) were used to assess the intra-observer and inter-observer reproducibility in group 1. In group 2, QAI values were correlated with the degree of hepatic steatosis using Spearman rank correlation analysis. Tentative cut-off values for steatosis were calculated with ROC analysis as compared to liver histology.

Results: For the intra-observer reproducibility of QAI, the ICC was 0.932 (95%CI, 0.854-0.969); inter-observer reproducibility showed an ICC of 0.902 (95%CI, 0.793-0.955). QAI measurements showed a significant correlation with the presence of hepatic steatosis (ρ 0.800; $p < 0.001$). QAI enabled the identification of mild steatosis ($S \geq 1$) with an AUC of 0.95 (95% CI:0.88-1.00) with an optimal cut-off of 0.61 dB/cm/MHz (sensitivity 93%; specificity 91%). The ROC values for differentiating significant steatosis ($S \geq 2$) from steatosis degree of less than S_2 was 0.97 (95% CI:0.94-1.00), with an optimal cut-off value of 0.72 dB/cm/MHz (sensitivity 84%; specificity 99%).

Conclusion: Attenuation imaging with QAI showed high intra- and inter-observer reproducibility in healthy volunteers. Correlation between QAI and hepatic steatosis assessed by standard US and liver histology is very good. Our study identifies for the first-time normal values of QAI in healthy volunteers and preliminary cut-off thresholds for steatosis staging in patients with metabolic liver disease.

P07-10-YI Double-blinded randomized controlled trial assessing the effect of consecutive fecal microbiota transplantation (FMT) on hepatic steatosis in patients with non-alcoholic fatty liver disease (NAFLD)

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Background and aims: The increasing prevalence of non-alcoholic fatty liver disease (NAFLD) poses a major burden on patients and healthcare systems. Besides lifestyle changes, there is currently no treatment available for NAFLD. This double-blinded randomized controlled trial assesses the effect of consecutive fecal microbiota transplantation (FMT) on hepatic steatosis as a potential treatment strategy for NAFLD.

Method: We recruited patients with NAFLD, diagnosed by ultrasound or VCTE FibroScan, from hepatology outpatient clinics of the Leiden University Medical Center (LUMC) and affiliated hospitals. Participants were randomized 1:1 to three times (t = 0; t = 3; t = 6 weeks) autologous or allogeneic FMT, performed directly into the duodenum during gastroscopy. FMT donor material was derived from two different donors (1:1) containing a stable, highly diverse and butyrate-rich microbiome. We assessed the change in hepatic steatosis, measured using MRI-PDFF, and the effect on liver biochemistry over a period of 12 weeks.

Results: In total, 20 patients participated (10:10). We found no significant change in MRI-PDFF in patients receiving allogeneic (18.6% (SD 9.1%) to 17.7% (SD 9.8%) (p = 0.37)) or autologous FMT (15.7% (SD 8.4%) to 15.4% (SD 7.4%) (p = 0.59)) (between-group difference: -0.54%, p = 0.63) after 12 weeks. Triglycerides decreased over time after allogeneic FMT (coeff: -0.46 (95% CI: -0.90;-0.017), p = 0.042) compared to autologous FMT, whilst no difference in effect was observed in ALAT, ASAT, AF and gGT.

Conclusion: Triple allogeneic FMT significantly decreased plasma triglycerides in patients with NAFLD over the course of 12 weeks, but did not affect hepatic steatosis. No effect on ALAT, ASAT, AF or gGT could be observed.

P07-12-YI Predictors of liver stiffness changes in a consecutive cohort of patients with NAFLD and longitudinal follow-up

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Background and aims: The serial use of non-invasive fibrosis tests can refine prognosis in patients with NAFLD and evaluate the progression or improvement of liver fibrosis. We evaluated predictors of improvement or worsening of liver stiffness values in a well characterized cohort of patients with NAFLD.

Method: We included a consecutive cohort of 263 patients with at least two outpatient visits between 2014 and 2022. The minimum time interval between baseline and follow-up LSM was >6 months. LSM worsening was defined as an increase of >20% kPa if the baseline LSM was ≥5 kPa, or a follow-up LSM >6 kPa if the baseline LSM was <5 kPa. An LSM improvement was defined as an LSM decrease of >20% kPa (if the baseline LSM was >6 kPa). A significant change in weight was defined as a >5% reduction or increase at follow-up, while a significant change in HbA1c was defined as a >10% reduction or increase. The variation between the true and expected FIB-4 index (based on the patient's age at the follow-up visit, but using the blood tests performed at the first visit) was calculated. A significant improvement or worsening in FIB-4 was defined as a >20% variation between the actual and "expected" FIB-4.

Results: Of the 263 patients, 161 (61%) were males; mean age was 54 ± 13 years. The median time from the first visits was 21.5 (14-30) months. 104 and 45 patients had an LSM >8 kPa and LSM >12 kPa at follow-up, respectively. 70 patients (26.6%) had an LSM improvement, while 55 (20.9%) had an LSM worsening; 133 patients (50.6%) maintained a stable value. In patients with an LSM improvement, 21 had an improvement, 15 had worsening, while 31 had stable FIB-4. In patients with an LSM worsening, 9 had an improvement, 21 had worsening, while 22 had a stable FIB-4. In multiple logistic regression, LSM worsening was independently associated with longer follow-up interval time, and an increase in the AST levels (OR 1.03 and 1.01 respectively) while LSM improvement was independently associated with weight reduction (OR 0.95) and HbA1c improvement (OR 0.98).

Conclusion: Approximately 50% of patients with NAFLD have significant changes in their LSM measurements over a period of 20 months, with worsening or improvement at equal rates. Improvement in metabolic comorbidities was independently associated with significant improvement in LSM measurements, further supporting a multidisciplinary model of care.

Figure: Multivariate analysis for Fibroscan LSM increase and for LSM decrease.

Variable	Multivariate for LSM worsening		Multivariate for LSM improvement	
	OR (95% CI)	p value	OR (95% CI)	p value
Age	-	-	1.02 (0.99-1.05)	0.09
Weight change, %	-	-	0.95 (0.90-0.99)	0.03
BMI	1.04 (0.99-1.09)	0.14	-	-
DM	-	-	0.72 (0.39-1.33)	0.30
Follow-up time	1.03 (1.01-1.02)	<0.01	1.01 (0.99-1.01)	0.78
AST change, %	1.01 (1.00-1.02)	<0.01	0.99 (0.99-1.01)	0.74
ALT change, %	0.99	0.40	0.99 (0.99-1.01)	0.50
HbA1c change, %	1.01 (0.99-1.02)	0.30	0.98 (0.96-1.00)	0.05

P07-14 Phosphatidylcholines (36:1) and (36:3) are increased in postmenopausal women with NAFLD.

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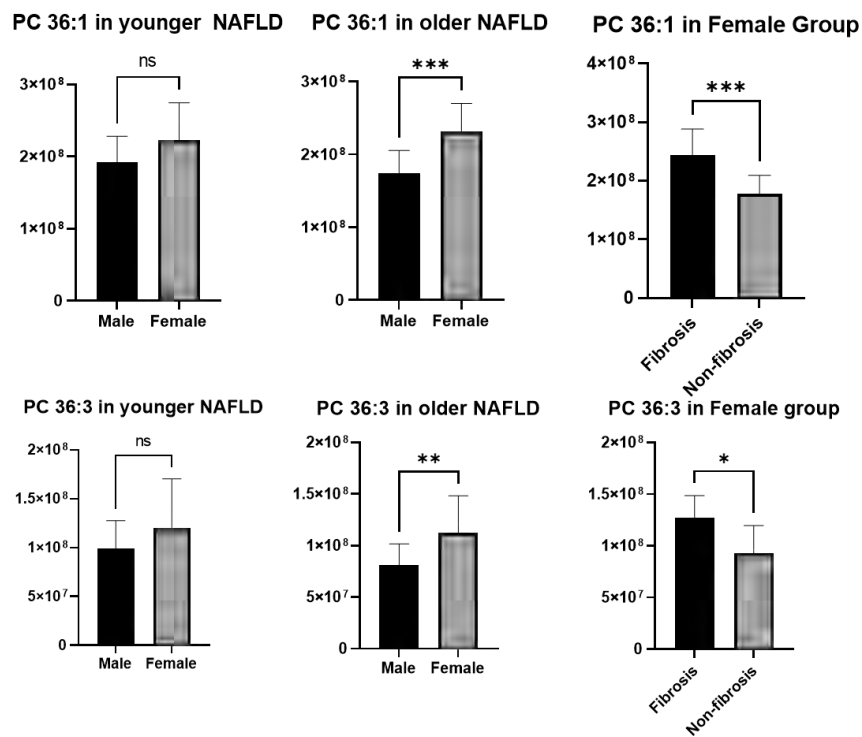
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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease, with a worldwide prevalence of 30%. Below the age of 50 years, there is a higher prevalence of NAFLD in men than in pre-menopausal women. However, in women, the prevalence of NAFLD increases after menopause with a rising trend observed above the age of 50 years, followed by a peak at 60 to 69 years, before declining after the age of 70 years. We aim to characterise the lipid profile in plasma and extracellular vesicles (EVs) in women of different age groups within our NAFLD cohort.

Method: Plasma samples were obtained from 60 NAFLD patients and 20 healthy controls. EVs were isolated via the Total Exosome Isolation Reagent (Thermo) from plasma and were identified via transmission electron microscopy. The lipids extracted from plasma and EVs were analysed via ultra-high performance liquid chromatography/ion mobility time-of-flight mass spectrometry (UHPLC/IM-QTOF-MS)-based untargeted lipidomics. Data was processed via a KniMet pipeline and R. Statistics analysis was performed via SIMCA and GraphPad Prism.

Results: 25 females and 35 males with a histological diagnosis of NAFLD were included. Female patients >55 years (x = 16) were considered as postmenopausal. In both EVs- and plasma- lipidomics analysis, the PCA, PLS regression and OPLS-DA models showed strong correlations between phosphatidylcholines (PC), age, gender and the diagnosis of NAFLD. PC (36:1) and PC (36:3) showed higher levels in NAFLD patients ≥55 years old compared to younger patients. The increase of PC (36:1) and PC (36:3) were also shown in female patients compared to male patients. Female patients with advanced fibrosis patients had more PC (36:1) and PC (36:3) compared to those without fibrosis. There was no difference in males.

Conclusion: Phosphatidylcholines (PC (36:1) and PC (36:3)) were abundant in postmenopausal females with NAFLD. And they are increased in NAFLD patients with fibrosis compared with NAFLD patients without significant fibrosis. These lipids might play a protective role in fibrosis progression in NAFLD.

Figure:

P07-15 Multiparametric liver ultrasound assessment for stratification of non-alcoholic fatty liver disease (NAFLD) severity: a possible role for viscosity

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Background and aims: Liver biopsy is the gold standard for grading and staging non-alcoholic fatty liver disease (NAFLD). In last few years many ultrasound-based non-invasive diagnostic tools have been developed. They showed high reliability to assess liver fibrosis (elastography) and liver steatosis (controlled attenuation parameter, CAP) but low accuracy in measure necro-inflammatory grade. Viscosity of liver tissue, measured through shear wave spectroscopy, has been recently proposed as a non-invasive tool for grading of necro-inflammatory activity in liver diseases. The aim of this study was to assess the potential role of liver Viscosity in stratification of NAFLD disease severity (Ballooning and lobular inflammation) and in diagnosis of non-alcoholic steatohepatitis (NASH).

Method: In this prospective study from November 2019 to December 2021, NAFLD patients who were about to undergo liver biopsy for Staging and Grading of disease were consecutively enrolled at Fondazione Policlinico A. Gemelli IRCCS in Rome. Liver biopsy was indicated for all patients with liver steatosis at ultrasound examination and either Fibroscan ≥ 8 kPa or high ALT and AST (≥ 2 upper normal limit) with metabolic comorbidities (diabetes, obesity and/or hypertension). Fibroscan, 2D-Shear wave elastography (2D-SWE) and Viscosity (Vi.PLUS) were performed on the same day of biopsy or within 3 months. 2D-SWE and Vi.PLUS were obtained with the Aixplorer ultrasound imaging system (Aixplorer-Mach30).

Results: 120 patients were enrolled. 68 were male (56, 7%) with mean age of 49 years. Obesity or diabetes were present in 72 (60%) and 35 (29, 1%) patients, respectively. Vi.PLUS showed a good diagnostic performance for lobular inflammation ≥ 2 (AUC 0, 71), for Ballooning ≥ 1 (AUC 0, 69), for the presence of both Ballooning ≥ 1 and lobular inflammation ≥ 1 (AUC 0, 70) and for NASH (AUC 0, 65). Vi.PLUS $\geq 2,3$ Pa.s has high specificity for diagnosis of both Ballooning grade ≥ 1 and lobular inflammation ≥ 1 (92, 9%), although low sensitivity (47, 2%). 2D-SWE and Fibroscan had similar diagnostic performance for advanced Fibrosis ($F \geq 3$) (AUC 0.90 and 0.93 respectively). 2D-SWE liver stiffness ≥ 7.2 had high specificity and high sensitivity for diagnosis of advanced fibrosis (88.2% and 81.4% respectively).

Conclusion: This study showed that Vi.PLUS is associated with both lobular inflammation and ballooning grade in NAFLD patients. Vi.PLUS could be used together with 2D-SWE to stratify non-invasively the risk of high NAFLD disease activity and advanced fibrosis. Nevertheless, clinical utility of Vi.PLUS remains uncertain due to low sensibility that keeps NASH hard to be excluded with non-invasive tests.

P07-17 NAFLD identification gap in italian primary care is due to a lack of proper ICD9 coding for disease

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Background and aims: The increasing prevalence of metabolic syndrome and obesity is increasing globally, and non-alcoholic fatty liver disease (NAFLD) has emerged as the most frequent cause of liver disease worldwide, accounting for approximately 25% of the adults and encompassing a variety of conditions from simple steatosis to end-stage liver disease. The purpose of our study was to assess the prevalence rate of NAFLD using data proceeds from the primary care services.

Method: The HSD database, a real-world dataset representative of each Italian geographical macro-area in terms of number of reference populations was used to retrospectively collect data of adults with NAFLD. Cases were identified via ICD9-CM diagnostic code (571.8) and the Hepatic Steatosis Index (HSI) >36.

Results: During the study period 918, 954 active individuals were identified. NAFLD was present in only 83, 981 (9.14%). Among NAFLD cases 18, 309 were identified using the ICD-9-CM diagnostic code, and 65, 672 had an HSI score >36.

Conclusion: This large real-world study identified more than 94% of the NAFLD cohort using HSI. This observation indicates that this is an undetected and/or underreported condition in many cases, which could also be attributed to the lack of a specific ICD-9-CM code, and thus primary care physicians may face difficulties in recording data and identifying patients properly.

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P07-20 Fatty liver disease in ALPHA-1 deficiency patients

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Background and aims: Alpha-1 Antitrypsin Deficiency (AATD) is a common, inherited disorder. The most severe form is seen in individuals with the homozygous PiZZ variant, with almost no circulating levels of AAT, while heterozygous (PiMZ; PiSZ) variants are associated with milder disease. While AATD effects on the lung have been extensively studied, its effects on the liver are poorly understood. Transient Elastography is widely used to stage liver disease by liver stiffness measurement (LSM), and provides a measure of hepatic steatosis, the Controlled Attenuation Parameter (CAP).

Method: Clinical data, TE measurements and blood tests were taken on adult patients presenting to a dedicated AATD clinic over a 12-month period.

An abnormal ALT level was >41 IU/L as per local lab. LSM cutoffs used were >7.1kPa for fibrosis, and >10kPa for advanced fibrosis/cirrhosis. CAP cutoffs for fatty liver were >250 dB/m

Results: 170 patients were recruited. 92/170 (54%) had a CAP >250dB/m indicating fatty liver. Of them, 51 had ALT measurements available, and 12 (23.5%) had a raised ALT. 78/170 were PiZZ (46%), 6 were PiSS. 54/170 (32%) were PiMZ, 23 PiSZ (13.5%) and 3 were PiMS. 47/170 had LSM >7.1kPa (27%).

66/92 (71%) of those with fatty liver disease also had a raised LSM score as well. This seemed more common with PiZZ, 17/39, (43.5%) as compared to PiMZ, 10/35, (28.5%), and PiSZ (25%). In contrast, fatty liver appeared more pronounced in milder AATD phenotypes; the median CAP for PiZZ individuals was 259 dB/m, for PiSZ was 267 dB/m and for PiMZ was 279 dB/m.

48 individuals had a BMI >30kg/m², of them 35 (73%), had a high CAP of >250. 16 (33%) of these individuals also showed a high LSM score

Conclusion: In this study, a high rate of previously undiagnosed concomitant fatty liver was found, affecting more than 50% of AATD patients. ALT levels did not reflect fatty liver. While a higher proportion of PiZZ individuals had raised LSM score compared with other phenotypes, median CAP scores were less in the more severe AATD phenotype. The contribution of concomitant fatty liver disease in AATD merits further investigation.

P07-21 Molecular mechanisms involved in metabolic dysfunction-associated fatty liver disease in cholecystectomized patients

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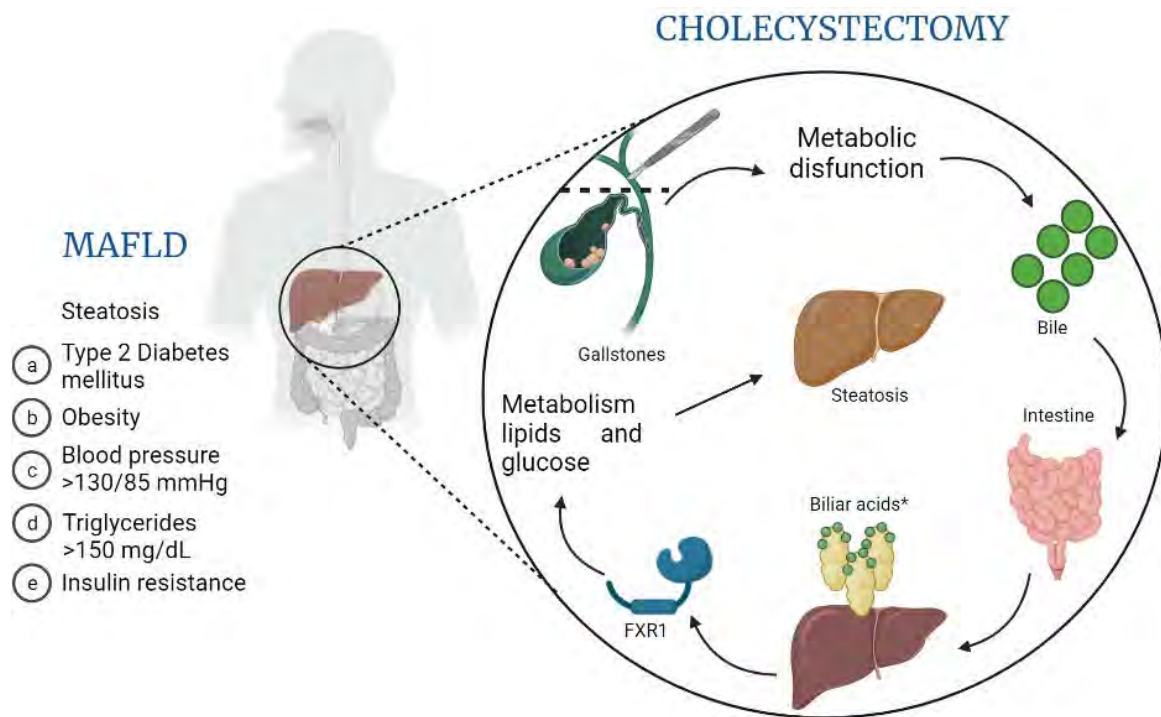
Background and aims: Gallstone disease and metabolic dysfunction-associated fatty liver disease (MAFLD) have a wide array of shared risk factors within their development, as well as interlinked pathologic mechanisms such as insulin resistance. There's an independent relationship between cholecystectomy and MAFLD development, and a recent study showed that patients with long-standing cholecystectomy (≥ 6 months) are at increased risk of severe liver fibrosis and cirrhosis at the time of MAFLD diagnosis. A wide array of molecules is involved in MAFLD progression, including farnesoid X receptor 1 (FXR1) and fibroblast growth factor receptor 4 (FGFR4) (Fig.1). The aim of this study was to explore the molecular mechanisms in fibrosis development in cholecystectomized MAFLD patients.

Method: We carried out a study analyzing 12 liver biopsies taken from Médica Sur Hospital in Mexico City during programmed cholecystectomies, in order to evaluate the expression FGFR4 and FXR1. The expression of these genes was calculated through qPCR analysis on the RNA of biopsied liver tissues. Furthermore, all patients included received 1, 3 and 6- months follow-up where metabolic biochemical markers were measured (liver function test, glucose, insulin, HbA1c, and lipid profile), as well as elastography, as indirect fibrosis indicators.

Results: There was strong correlation between the expression of FGFR4 and steatosis degree ($r = 0.779$, $p = 0.023$), ballooning degeneration ($r = 0.764$, $p = 0.027$), interphase inflammation ($r = 0.756$, $p = 0.030$) and steatosis activity score (SAS) ($r = 0.779$, $p = 0.023$). Furthermore, FXR1 expression through qPCR did not have any significant association with any type of histological hepatocyte injury. Serum triglycerides had a significant change among patients with steatosis and those with steatohepatitis ($p = 0.036$), as well as serum insulin levels; however, other metabolic biochemical markers remained without significant change between patients previous to cholecystectomies and in their 6-month follow-up period. Among the participants, one of the patients progressed in fibrosis degree after the cholecystectomy (from F1 to F2). Compared to the others, this case presented similar FGFR4 expression at the moment of the surgery; however, basal metabolic indicators varied widely: BMI >35 , total bilirubin >2.0 , AST >600 , GGT >400 , showing more than 200% increase compared to the cases where fibrosis did not progress in the 6-month period.

Conclusion: In conclusion, progression of fibrosis after cholecystectomy was associated not with increased FGFR4 expression, but rather with significantly worse metabolic profile at the moment of cholecystectomy. We found strong correlation linking FGFR4 expression with ballooning degeneration, interphase inflammation, and SAS, pointing towards its role in lipid and glucose metabolism.

Figure:



P07-22 Promising ultrasound tools in the management of patients with NAFLD

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Background and aims: Auto Point Shear Wave Elastography (auto-pSWE) is a new liver stiffness quantification tool designed to reduce liver elastography exam time. Ultrasound Derived Fat Fraction (UDFF) is a new measurement tool to assess hepatic steatosis. The aim of the study was to assess the diagnostic accuracy of conventional pSWE, auto-pSWE and UDFF.

Method: Patients with chronic liver disease who had already performed liver biopsy were consecutively recruited from our outpatient department to participate in this cross-sectional study. Conventional pSWE (obtaining 10 measurements through 10 acquisitions), auto-pSWE (automatically obtaining 15 measurements by a single acquisition), and UDFF (one measurement obtained by one acquisition) of the liver were performed using the Acuson Sequoia ultrasound system between March and May 2022.

Results: A total of 110 patients were included, the majority female (50.9%), with a median age of 55 years old (IQR 46-62). The Pearson correlation coefficient between UDFF and histologic steatosis was 0.487 ($p < 0.001$). The UDFF identified patients with a higher probability of having histologic steatosis: the area under the receiver operating characteristic curve (AUROC) values for diagnosing steatosis >grade 0 was 0.788 (95%CI 0.695-0.881, $p < 0.001$), for steatosis >grade 1 was 0.827 (95%CI 0.745-0.909, $p < 0.001$) and for steatosis >grade 2 was 0.728 (95%CI 0.618-0.837, $p < 0.001$). The Pearson correlation coefficient between pSWE and auto-pSWE was 0.505 ($p < 0.001$). The AUROCs for diagnosing fibrosis stage >1, >2 and >3 was 0.691 (95%CI 0.580-0.802, $p = 0.001$), 0.789 (95%CI 0.662-0.917, $p < 0.001$) and 0.888 (95%CI 0.789-0.987, $p < 0.001$) for pSWE and 0.674 (95%CI 0.566-0.783, $p = 0.002$), 0.740 (95%CI 0.611-0.869, $p = 0.001$) and 0.724 (95%CI 0.539-0.910, $p = 0.020$) for auto-pSWE, respectively.

Conclusion: UDFF tool provides a simple, non-invasive and low-cost tool for quantifying the hepatic fat fraction with a high degree of agreement with histologic biopsy. The auto-pSWE has equal accuracy as conventional pSWE in measuring liver stiffness, with the advantage of time effectiveness.

P07-23 Development of fatty liver disease in the portuguese population: can we predict it?

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Background and aims: The impact of non-alcoholic fatty liver disease (NAFLD) is increasing worldwide, along with obesity and metabolic syndrome (MS) pandemics. Nowadays, NAFLD represents one of the most common causes of advanced liver disease and transplant in Europe. This study aims to evaluate fatty liver disease (FLD) incidence and prevalence in a Portuguese cohort, after a 10-year period, within the scope of e_COR study. The e_COR study was promoted by National Institute of Health in 2012, designed to assess the prevalence of cardiovascular disease risk factors in the Portuguese adult population.

Method: From the previous cohort of 214 patients, 63 patients were contacted again to reevaluate their clinical evolution. Clinical and anthropometrical data, hepatic steatosis and fibrosis grades, as well as cardiovascular and oncological outcomes were collected.

Results: The initial cohort of 214 participants in 2012 presented FLD prevalence of 38.5%. In 2022, 63 patients (29.4%) were reevaluated, with mean age of 57.7 ± 16.3 years; most male (52.4%). During follow-up (FU), FLD was present in 31 of 63 cases (51.7%). Liver elastography mean values in the two periods of this new cohort were 4.9 ± 1.3 kPa vs. 4.3 ± 0.8 kPa ($p = 0.042$). From the 46 patients (73.0%) with previous non-fatty liver, 18 (41.9%) developed FLD. From the 16 individuals with FLD (25.4%), only 4 (6.3%) managed to resolve their clinical condition ($p = 0.024$). In univariate analysis, we identified as risk factors for FLD incidence the body mass index, abdominal circumference during FU ($p < 0.001$), MS development ($p = 0.041$), glycated haemoglobin ($p = 0.037$) and obstructive sleep apnea syndrome (OSAS) risk through the STOP-Bang questionnaire ($p = 0.017$). During FU, 11 patients (17.5%) developed neoplasia (12.7%) or acute myocardial infarction (4.8%) with no correlation with metabolic syndrome or FLD ($p = 0.315$) in this cohort.

Conclusion: The preliminary results of this study demonstrate that is an increasing incidence of FLD as patients grow old. Identification of risk factors as MS, glycated haemoglobin and OSAS risk allows us a holistic approach of FLD in multidisciplinary teams, that may attenuate this phenomenon.

P07-24 Performance of fatty liver index in the prediction of advanced chronic liver disease in non-alcoholic fatty liver disease

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Background and aims: Nowadays, Non-alcoholic Fatty Liver Disease (NAFLD) is a public health problem. Its prognosis essentially depends on the degree of hepatic fibrosis. Fatty Liver Index (FLI), a simple score originally proposed to predict fatty liver disease in the general population, may have a prognostic value in NAFLD (1).

Our objective was to evaluate the performance of FLI in the assessment of fibrosis in NAFLD.

Method: This prospective study included consecutive patients followed at our outpatient clinic from January 1, 2021, to September 30, 2021. Liver elasticity was assessed by Fibroscan. FLI and FIB-4 scores were calculated. Advanced chronic liver disease was defined by liver elasticity >9.6 kpa and FIB-4 >2.67.

Results: Ninety-nine patients were collected. The mean age was 55.49 ± 11.91 years and the sex ratio (M/F) was 0.5. The average body mass index was 32.37 kg/m^2 . Patients had a history of diabetes in 46.3% of cases and high blood pressure in 40.4% of cases. A significant correlation was noted between the FLI score and hepatic stiffness assessed by Fibroscan ($p = 0.001$). FLI score was also significantly correlated to the FIB-4 score ($p = 0.023$). At a cutoff of 82, the sensitivity and specificity of FLI score in predicting advanced chronic liver disease in NAFLD were 70.6% and 67%, respectively. The area under the ROC curve of FLI in the prediction of advanced chronic liver disease was 0.72.

Conclusion: In our study, the FLI score seems to have a good predictive value of advanced chronic liver disease in NAFLD. Strongly correlated with the Fibroscan and the FIB-4 score, the use of this score can consolidate their contributions in the assessment of fibrosis in NAFLD.

References: 1- Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol. 2006 Nov 2;6:33.

**POSTER
ABSTRACT
PRESENTATION**

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P03-07 Assessment of liver fibrosis with transient elastography in NAFLD patients

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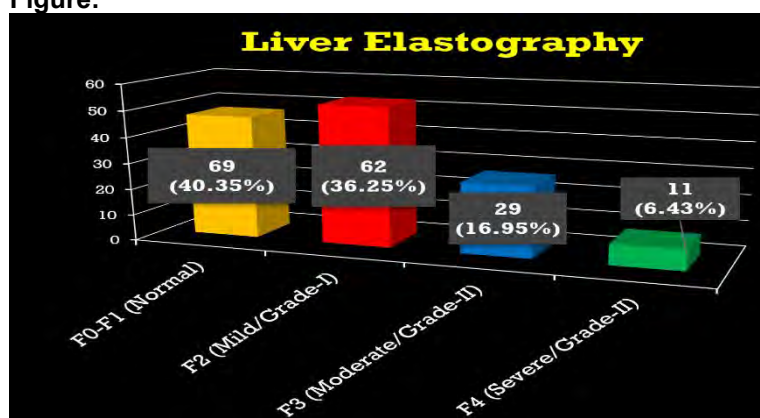
Background and aims: Transient Elastography (TE) is a non-invasive technique for estimating liver fibrosis. There is a limited data about the performance of TE in Pakistani patients with non-alcoholic fatty liver disease (NAFLD). NAFLD is a global outbreak, it is pivotal that patients with NAFLD should undergo an assessment for their risk of advanced fibrosis, which enhances the risk of hepatocellular carcinoma (HCC) and other complications of cirrhosis. An overall prevalence of NAFLD in Pakistan is 47%. In the present study, we have evaluated the diagnostic accuracy of TE in identifying different degrees of fibrosis in NAFLD adult patients

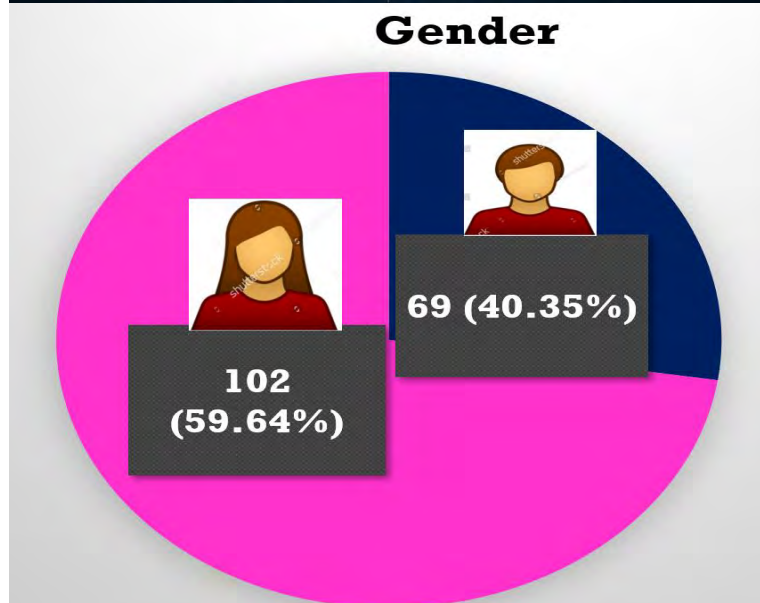
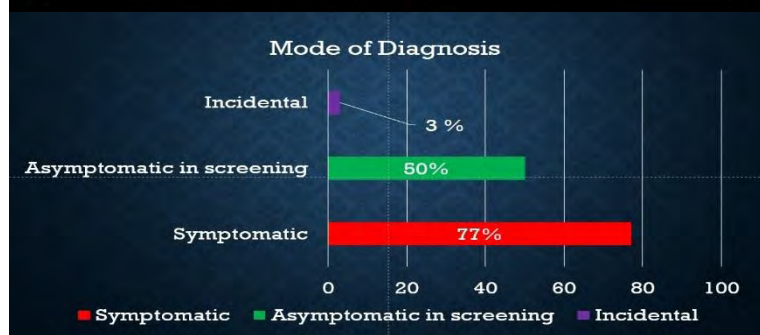
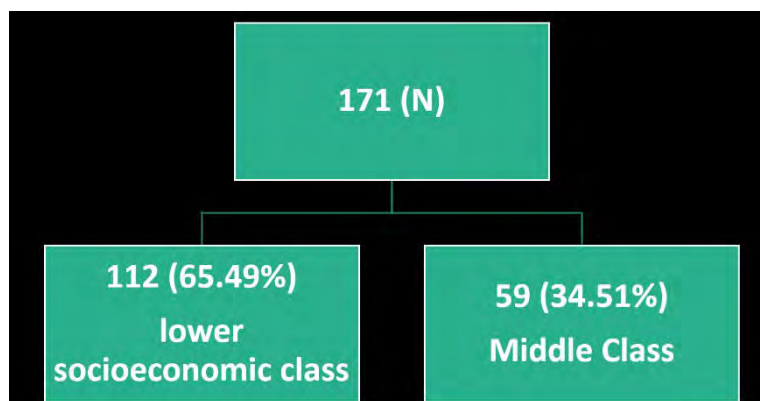
Method: A Cross-sectional study was undertaken at the Department of Gastroenterology, Jinnah Postgraduate Medical Centre and Medical Unit II, Dow University of Health Sciences Ojha campus Karachi, Pakistan. After obtaining ethical approval, all patients above the age of 18 years, with diagnosis of NAFLD on the basis of abnormal liver function tests (LFTs) and on ultrasound abdomen consistent with fatty liver were included in the study. All patients with hepatitis, hepatic malignancies, hepatobiliary infections, and biliary tract disease were excluded from the study. Fibrosis score was calculated through Elastography as: F0-F1 (5.3-7.1 kPa, Normal); F2 (7.5-8.5 kPa, Mild/Grade-I); F3 (9.5-13.0 kPa, Moderate/Grade-II); and F4 (13.1-18.8 kPa, Severe/Grade-III). This study is an ongoing study.

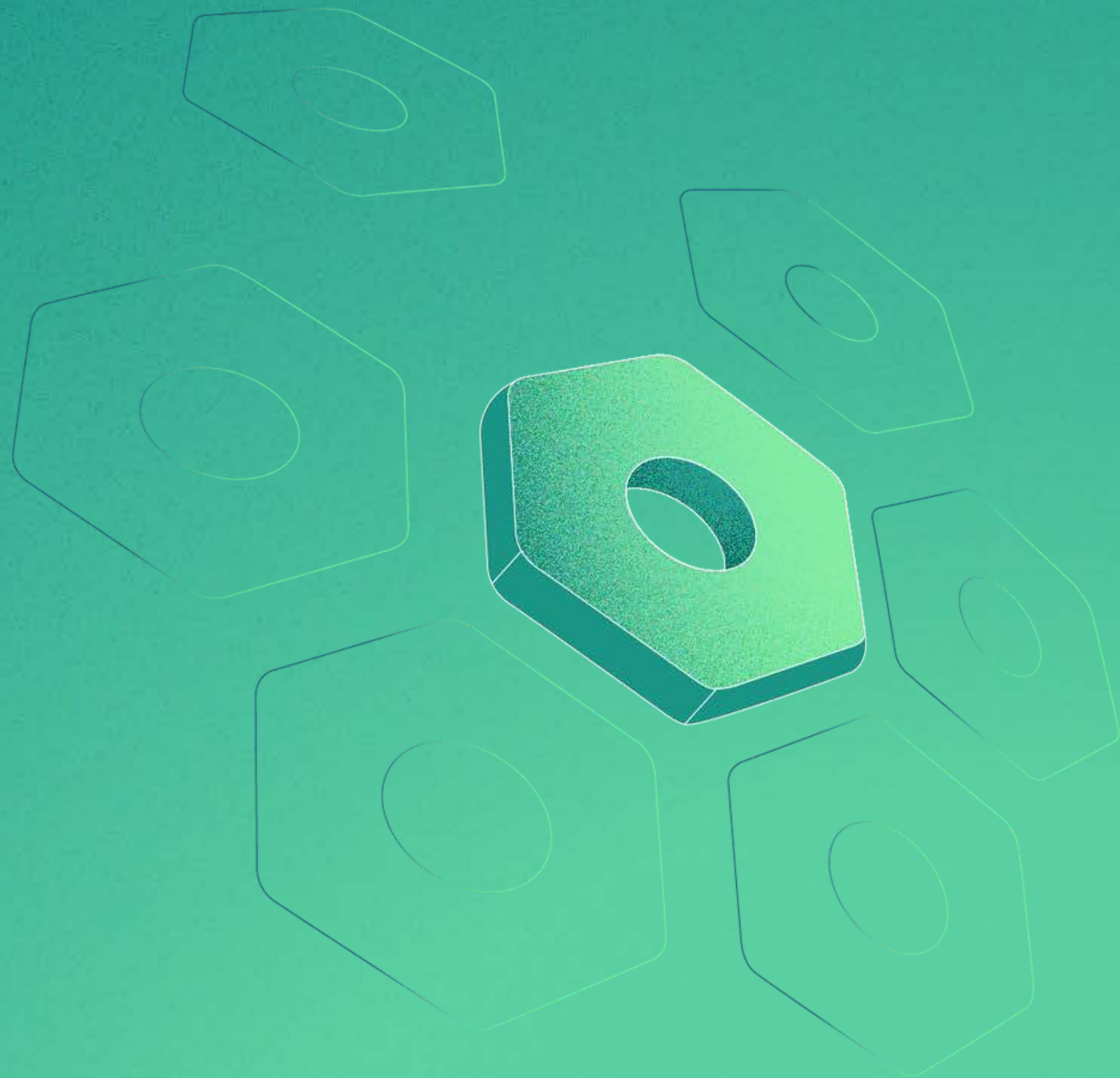
Results: A total of 171 patients were enrolled in the study, from which 69 (40.35%) were male and 102 (59.64%) were female, with a mean age of 37.50 ± 9.74 years. Out of these, 112 (65.49%) belonged to the lower socioeconomic class. One hundred and twenty two (71.34%) patients had fatty liver on ultrasound and 49 (28.65%) had hepatomegaly with fatty changes. TE revealed 69 (40.35%) patients had a score of F0-F1, 62 (36.25%) F2, 29 (16.95%) F3, and only 11 (6.43%) had a score of F4.

Conclusion: The detection of liver fibrosis at early stages is crucial in preventing its progression to cirrhosis which is the irreversible process. Reversal of fibrosis is only possible if it is diagnosed as early as possible and managed with appropriate treatment.

Figure:







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