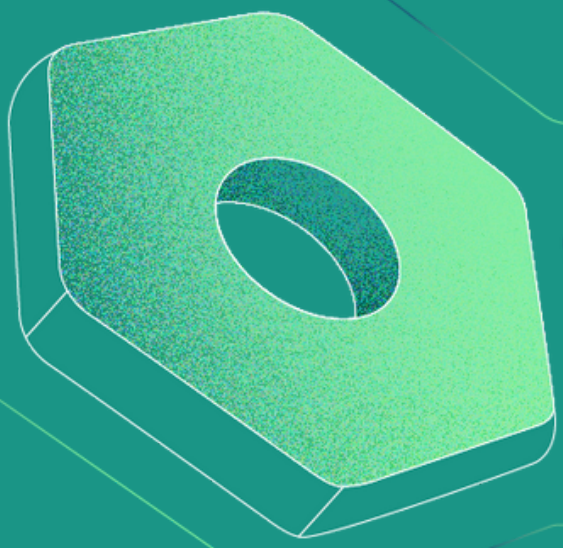
EASL SLD SUMMIT 2023

Prague, Czechia
21–23 Sept

ABSTRACT BOOK



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ORAL ABSTRACT PRESENTATIONS

OS-1-YI
NK cells from steatotic liver disease patients exhibited high NTCP expressions associated with STAT signaling pathway and impaired function

Johnny Amer[1], Ahmad Salhab[1], Rifaat Safadi[1]
[1]*Hadassah-Hebrew University Hospital, Liver Institute, Jerusalem, Israel*
Email: johnnyamer@hotmail.com

Background and aims: NK cells play important roles in innate defences and mediate an antifibrotic effect in the early stages of chronic liver disease and their function are thought to be impaired in advanced liver diseases. The regulation/induction of NK cell function is mediated by an array of activating or inhibitory surface receptors. Our study aimed to assess the impact of bile acids signaling pathway on NK functions and changes in their molecular aspects mediated by NTCP in patients with steatotic liver disease (SLD).

Method: Human peripheral and tissue-resident NK cells from adult patients (age ≥18 years) of histologically documented SLD with different Metavir scores of liver fibrosis were obtained. Healthy volunteers were also recruited. Molecular aspects of NTCP expressions, STAT signaling, NK activatory markers [NKp46, NKp30, CD107], exhaustion markers [PD1, TIGIT, LAG-3]. Bile acid signaling pathways [modulated using agonists and antagonists], metabolic profiles and trNK phenotypic alterations were assessed in mice models of (1) liver fibrosis (carbon tetrachloride), (2) leptin deficient mice (*Ob/Ob*) with high fat diet [*Ob/Ob*^{HFD}] and (3) adoptive transfer models of immune alterations through NK cells transplantation following sorting according to their distinct NTCP expressions were used.

Results: Data obtained showed that peripheral blood and liver tissue-resident NK cells obtained from F3/F4-scored patients of liver fibrosis exhibit 2.5-fold increased NTCP expressions compared to F0-scored patients and healthy volunteers. Sorted-NK cells (NK^{NTCP+}) showed high expressions of NTCP and high TCA uptake in-vitro and triggered a further increase in their exhaustion and impairment. Sorted NK^{NTCP+} cells showed high-phosphorylated pathways of STAT3 and low phosphorylated pathways of STAT5. Moreover, NK^{NTCP+} cells treated with STATIC; STAT3 inhibitor significantly decreased NTCP expression, decrease NK cells exhaustion and increase NK cells activity. EGCG (NTCP inhibitor) while inhibited NTCP (less TCA uptake), it attenuated liver fibrosis in both CCl_4 and *Ob/Ob*^{HFD} animal models with ameliorated metabolic profile. Moreover, trNK^{NTCP-} transplanted to immunosuppressed mice exhibiting liver fibrosis and fed with high fat diet (HFD) alleviate liver fibrosis through reductions in pro-inflammatory (TNF-a, IL-1b, IL-6 and IL-10) and pro-fibrotic (IL-4 and MCP-1) cytokines and amelioration lipid profile in the mice group ($p < 0.05$). Moreover, IL-2 and INF-g showed substantial increase in the mice receiving the trNK^{NTCP-} cells.

Conclusion: The study describes a novel inhibitory NK cell checkpoint mediated via elevation in NTCP expressions. NTCP upregulation regulate NK STAT signaling pathways and mediate NK exhaustion and impairment. Modulating NTCP expression or activity could be explored as a potential strategy to enhance NK cell-mediated immune responses, particularly in conditions where NK cell function is compromised or dysregulated.

OS-2
Characteristics and long-term mortality of individuals with MASLD, MetALD, and ALD, and the utility of SAFE score

Pimsiri Sripongpun[1 2], Apichat Kaewdech[1], Prowpanga Udompap[3], W. Ray Kim[2]
[1]Prince of Songkla University, Internal Medicine, Hat Yai, Thailand, *[2]Stanford University School of Medicine, Division of Gastroenterology and Hepatology, Redwood City, United States*, *[3]Mayo Clinic, Division of Gastroenterology and Hepatology, Rochester, United States*
Email: pim072@hotmail.com

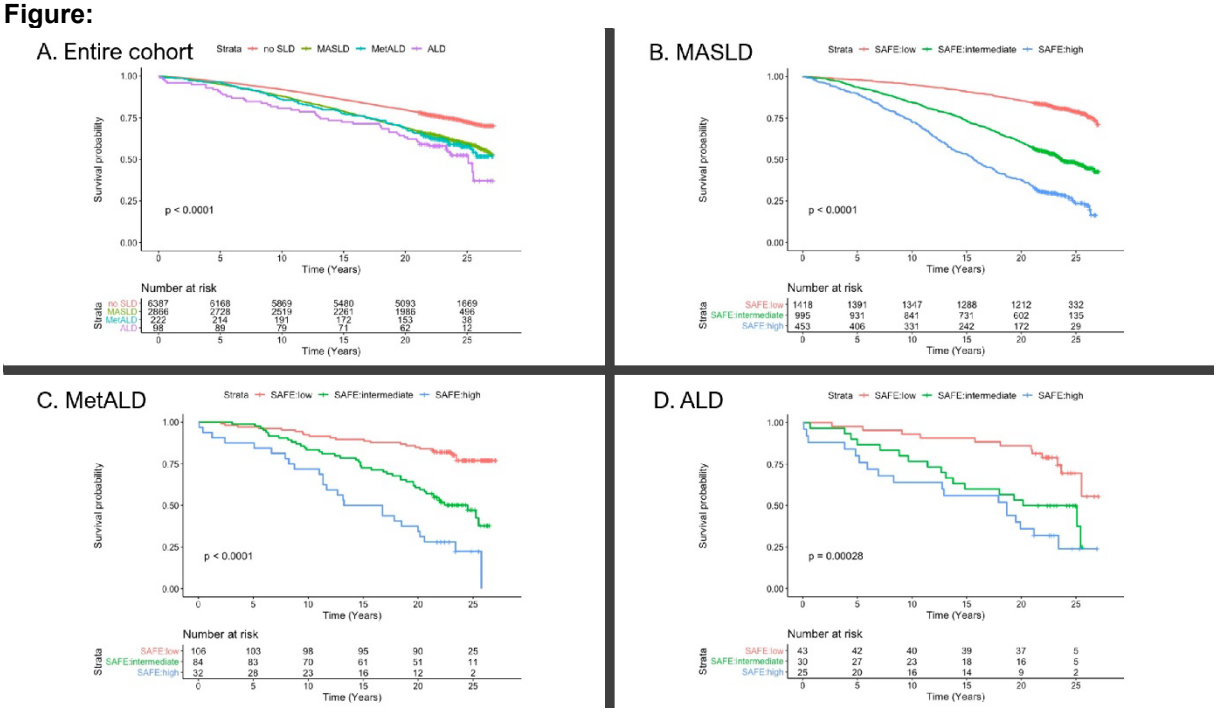
Background and aims: The new nomenclature of steatotic liver disease (SLD) has been recently launched. SLD's sub-classifications include metabolic dysfunction-associated SLD (MASLD), MASLD and increased alcohol intake (MetALD), and Alcohol-related liver disease (ALD). It is unknown whether these new subgroups of patients have different characteristics and long-term outcomes as well as the performance of non-invasive biomarkers in these subgroups. Specifically, we correlated the recently developed steatosis-associated fibrosis estimator (SAFE) score with long term survival of subjects with SLD.

Method: Using the National Health and Nutrition Examination Survey (NHANES) III data (conducted in 1988-1994) and their linked mortality data (censored on December 31, 2015), we selected all adult participants who had available ultrasound description of their liver with regard to steatosis. Exclusion criteria were those with hepatitis B or C, no data on alcohol consumption and cardiometabolic risk, and those without available data on variables to calculate the SAFE score. The characteristics between those without SLD (no steatosis on ultrasound), MASLD (at least 1 of the following: BMI≥25, waist circumference≥80F/90M, diagnosis of hypertension, diabetes, or hyperlipidemia and alcohol <20F/30M gm/day), MetALD (at least 1 metabolic criteria and alcohol 20-50g/d in women and 30-60g/d in men), and ALD (alcohol ≥50g/d in women and 60g/d in men with or without cardiometabolic risk) were compared. The overall survival (OS) was described using the Kaplan-Meier methods for low (SAFE<0), intermediate (SAFE 0~100), and high (SAFE≥100) risk strata for SLD subgroups.

Results: Of 17, 295 NHANES III participants, 9, 573 were eligible for this analysis. Sixty-seven percent of the participants had no SLD, while 29.9%, 2.3%, and 1% were in MASLD, MetALD, and ALD groups, respectively. Among those with SLD, a higher proportion of male sex was observed in MetALD and ALD than in MASLD (71.6% vs 86.7% vs 53.3%, $p < 0.001$, respectively) as well as active smoker (42.8% vs 54.1% vs 22.6%, $p < 0.001$, respectively). Diabetes was more prevalent in MASLD than in MetALD or ALD (19.9% vs 13.5% vs 9.2%, $p = 0.003$, respectively). Figure shows the OS of those 4 subgroups. In Figure 1a, among those with SLD, ALD subgroup showed a significant lower OS than those with MASLD ($p = 0.049$), but the OS of the MetALD subgroup was not significantly different from that of MASLD ($p = 0.51$). Furthermore, SAFE score strata can differentiate the OS of all SLD subgroups significantly (Figures 1b-d).

Conclusion: In general, the proportion of those with MASLD was far more common than MetALD and ALD. MetALD shared the characteristics of both MASLD and ALD subgroups. Those with ALD experienced significantly lower OS than that in MASLD, while there was no significant between MetALD and MASLD subgroups. SAFE score may be used as a non-invasive test to stratify long-term risk in all 3 SLD subgroups.

Figure:


A. Entire cohort
Strata no SLD MASLD MetALD ALD
Survival probability
p < 0.0001
Time (Years)
Number at risk
no SLD 6387 6168 5869 5480 5093 1669
MASLD 2886 2728 2519 2261 1986 496
MetALD 222 214 191 172 153 38
ALD 98 89 79 71 62 12
B. MASLD
Strata SAFE:low SAFE:intermediate SAFE:high
Survival probability
p < 0.0001
Time (Years)
Number at risk
SAFE:low 1418 1391 1347 1288 1212 332
SAFE:intermediate 995 931 841 731 602 135
SAFE:high 453 406 331 242 172 29
C. MetALD
Strata SAFE:low SAFE:intermediate SAFE:high
Survival probability
p < 0.0001
Time (Years)
Number at risk
SAFE:low 106 103 98 95 90 25
SAFE:intermediate 84 83 70 61 51 11
SAFE:high 32 28 23 16 12 2
D. ALD
Strata SAFE:low SAFE:intermediate SAFE:high
Survival probability
p = 0.00028
Time (Years)
Number at risk
SAFE:low 43 42 40 39 37 5
SAFE:intermediate 30 27 23 18 16 5
SAFE:high 25 20 16 14 9 2

OS-3
Presence of portal hypertension in the absence of advanced fibrosis in patients with non-alcoholic fatty liver disease/metabolic dysfunction associated steatotic liver disease

Caroline Geens[1], Naomi Gestels[1], Lotte Schoenmakers[1 2], Zouhir Gadi[1 2], Jolien Derdeyn[1], Toon Steinhauser[1], Luisa Vonghia[1 2], Thomas Vanwolleghem[1 2], Sven Francque[1 2], Wilhelmus Kwanten[1 2]
[1]Antwerp University Hospital, Gastroenterology and Hepatology, Edegem, Belgium, *[2]University of Antwerp-Faculty of Medicine, Laboratory of Experimental Medicine and Pediatrics Division of Gastroenterology-Hepatology, Antwerpen, Belgium*
Email: caroline.geens@student.uantwerpen.be

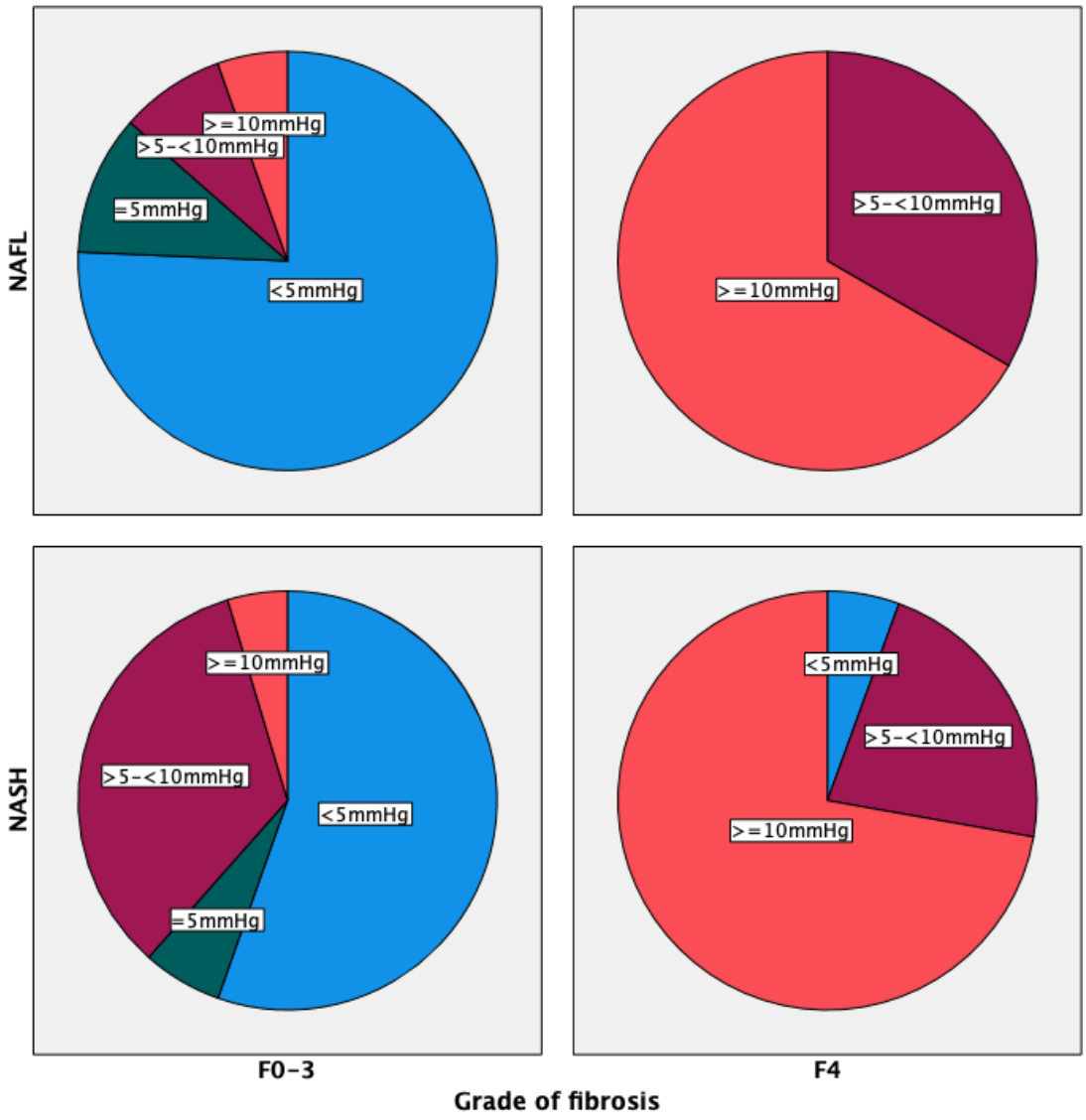
Background and aims: Portal hypertension (PH), defined as a hepatic venous pressure gradient (HVPG) >5 mmHg, is long believed to be restricted to chronic liver disease with advanced fibrosis. Recent data have shown that the portal venous pressure can be increased in early stages of non-alcoholic fatty liver disease (NAFLD) in which fibrosis is limited or even absent. We aim to study the prevalence and severity of PH amongst the spectrum of NAFLD.

Method: We retrospectively analysed HVPG measurements from 126 consecutive patients with NAFLD as single cause, who underwent both a HVPG measurement and a liver biopsy at our centre between February 2018 and May 2022. Liver biopsies were scored according to the Non-alcoholic Steatohepatitis Clinical Research Network (NASH-CRN) scoring system.

Results: Forty-three patients had NAFL and 83 patients had NASH; 55 (44 %) were women and 71 (56 %) were men; mean age was 58.17 ± 12.31 year (SD); mean BMI was 34.93 ± 7.62 kg/m^2. The criteria of Metabolic Dysfunction Associated Steatotic Liver Disease (MASLD) were met in 121 (96 %) patients. Mean HVPG in the NAFL group was 6.18 ± 6.41 mmHg and 6.94 ± 5.31 mmHg in the NASH group (ns). Fibrosis stage distribution was as follows F0-F1 51 (40 %), F2 27 (21 %), F3 24 (19 %) and F4 24 (19 %) patients. This distribution showed higher stages of fibrosis more frequently in patients with NASH compared to NAFL ($p < 0.001$). HVPG increased significantly with increasing stage of fibrosis ($p < 0.001$). Among the 78 NAFLD patients without advanced fibrosis (F0-F2), 14 (18 %) had PH (HVPG >5 mmHg) and 2 (2.6 %) had clinically significant PH (CSPH; HVPG $\geq$ 10 mmHg). In these 14 patients NAFL was present in 1 and NASH in 13 patients. Both cases with CSPH had NASH. Nine out of 14 (64 %) patients had F2 fibrosis, including both cases with CSPH. Among the 48 patients with advanced fibrosis (F3 and F4), 39 (81 %) had PH and 20 (42 %) had CSPH. NAFL was present in 11 and NASH in 37 out of the 48 patients; in those with CSPH these were 6 and 14 patients respectively. Interestingly, in those with F3 16/24 (67 %) had PH, of whom 3/24 (13%) had CSPH. As in cirrhosis the classical features of NASH tend to disappear, the relation between HVPG and histological features of NASH was assessed in the 102 non-cirrhotic patients (F0-F3). PH, and even CSPH, was observed in low grades of steatosis (S0-S1): 14/52 (27 %) had PH and 3/52 (5.8 %) had CSPH. In higher grades of steatosis PH was observed in 7/33 (21%) in S2 and 9/17 (53%) in S3 with CSPH in 1/33 (3%) in S2 and 1/17 (5.9%) in S3. Comparable results were obtained when the analysis was restricted to the 121 MASLD patients.

Conclusion: PH, and CSPH, as assessed by HVPG measurement, is observed in a substantial proportion of patients with early stage NAFLD. With increasing stages of fibrosis, prevalence and severity of PH increases. CSPH seems to be restricted to significant fibrosis and higher (≥F2). There is a very large overlap with MASLD, to which the same conclusions apply.

Figure:


NAFL
NASH
>=10mmHg
>5-<10mmHg
=5mmHg
<5mmHg
F0-3
F4
Grade of fibrosis

OS-4
Lean diabetic patients with steatotic liver disease have the same progression rate of hepatic fibrosis but increased weight gain compared to overweight ones despite less severe disease at baseline: a prospective follow-up study

Rosa Lombardi[1], Annalisa Cespiati[1], Alessandro Mantovani[2], Gabriele Maffi[1], Paolo Francione[1], Felice Cinque[1], Floriana Santomenna[1], Jaqueline Currà[1], Rosanna Villani[3], Claudio Maffeis[4], Antonio Colecchia[5], Nicola Passigato[6], Alberto Ferrarese[6], Caterina Cusumano[6], Emanuela Orsi[7], Valeria Grancini[7], Giuseppina Pisano[1], Daniela Bignamini[1], Giovanni Targher[2], Gaetano Serviddio[3], Silvia Fargion[1], Anna Ludovica Fracanzani[1]
[1]Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Unit of Internal Medicine and Metabolic Diseases, MILANO, Italy, *[2]University and Azienda Ospedaliera Universitaria Integrata of Verona, Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, VERONA, Italy*, *[3]University of Foggia, Centro C.U.R.E, Dept. of Medical and Surgical Sciences, FOGGIA, Italy*, *[4]University Hospital of Verona, Pediatric Diabetes and Metabolic Disorders Unit, Department of Surgical Sciences, Dentistry, and Pediatrics, and Gynaecology, VERONA, Italy*, *[5]University Hospital of Modena and Reggio Emilia, Gastroenterology Unit, Department of Medical Specialities, MODENA, Italy*, *[6]Azienda Ospedaliera Universitaria Integrata of Verona, Gastroenterology Unit, VERONA, Italy*, *[7]Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Department of Medical Science, Endocrinology and Diabetes Unit, MILANO, Italy*
Email: rosa.lombardi@unimi.it

Background and aims: Steatotic liver disease (SLD) is the accumulation of fat in the liver and often associates with metabolic alterations, configuring a state of metabolic associated steatotic liver disease (MASLD) and exposing patients to high risk of progressive liver damage. SLD can be present also in lean individuals but less is known about fibrosis progression in lean diabetics MAFLD ones. To prospectively evaluate change in hepatic fibrosis in lean diabetic MASLD patients versus overweight ones.

Method: 237 diabetic MASLD patients (mean age 67 ± 9 years, 54% male) were enrolled at the diabetology outpatient clinics and re-evaluated after 5 years. Information about diabetic control, metabolic comorbidities and medications were collected at baseline and follow-up. Hepatic steatosis was assessed by liver ultrasonography, fibrosis by Fibroscan® (liver stiffness measurement-LSM>8.2kPa). Genotyping for PNPLA3, TM6SF2 or MBOAT7 polymorphisms was available for all patients.

Results: Fifty (21%) patients were lean (BMI<25 kg/m2) and 187 (79%) overweight (BMI>25 kg/m^2), with no difference in age or sex. At baseline, lean vs overweight MASLD patients presented a lower prevalence of LSM >8.2 kPa (2% vs 11%, p = 0.05), with superimposable prevalence of dyslipidemia, hypertension and insulin-resistance. Similarly, no difference in the duration of T2DM nor in the glycemic control (HbA1c>7%, 50% vs 52% p = 0.874) was observed, neither in the type of basal antidiabetic therapy or genetics. During the follow-up period lean diabetic MASLD patients presented a superimposable rate of fibrosis progression by Fibroscan compared to overweight ones (LSM >30% from baseline 26% vs 18%, p = 0.226; median LSM worsening 1.0 kPa (0.1-12.6 kPa) vs 1.0 (0.1-11.8) p = 0.797), and this data was confirmed also when considering patients without baseline fibrosis (i.e LSM<8.2 kPa). Interestingly, lean diabetic MASLD subjects were more likely to gain weight (53.5% vs 35.5%, p = 0.031) over time compared to overweight diabetics, without any difference in the glycemic control (worsening of HbA1c 0.5% from baseline, 35% vs 48%, p = 0.255) or in the use of antidiabetic drugs at follow-up, including GLP-1agonists or SGLT2-inhibitors. Weight gain remained independently associated with worsening of LSM only in lean subjects but not in overweight ones (adjustment for age, sex and glycemic control; OR 4.3, CI 95% 1.0-8.6).

Conclusion: Over a 5-year follow-up diabetic MASLD patients presented a high progression of hepatic fibrosis, even if lean. Lean subjects seemed to weight gain more than overweight ones, and weight gain was an independent factor for progression of liver disease in this category of patients, independently of the glycemic control. Therefore, counseling about lifestyle is crucial especially in lean diabetic MASLD individuals in order to prevent weight gain and worsening of liver disease.

OS-5-YI
Zone-specific hepatic lipid metabolism in metabolic dysfunction-associated steatohepatitis

Patcharamon Seubnooch[1 2], Fabienne Birrer[2 3], Matteo Montani[4], Sofia Tsouka[1], Deborah Stroka[3], Jean-François Dufour[3 5], Mojgan Masoodi[1]
[1]Institute of Clinical Chemistry, Inselspital, Bern University Hospital, Bern, Switzerland, *[2]Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland*, *[3]Department of Visceral Surgery and Medicine, Inselspital, Bern University Hospital, University of Bern, Switzerland and Department for Biomedical Research, University of Bern, Bern, Switzerland*, *[4]Institute of Tissue Medicine and Pathology, University of Bern, Bern, Switzerland*, *[5]Centre des Maladie Digestives Lausanne, Lausanne, Switzerland*
Email: patcharamon.seubnooch@insel.ch

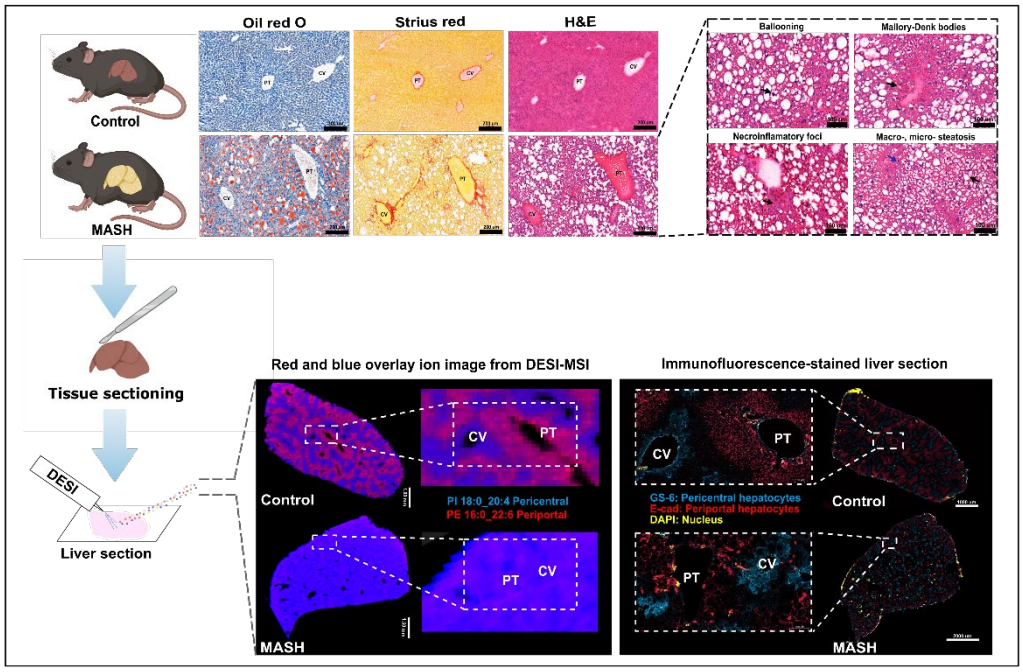
Background and aims: Alterations in lipid metabolism play a pivotal role in developing metabolic dysfunction-associated steatohepatitis (MASH). The liver displays gradient levels of oxygen and nutrients across its lobules, resulting in distinct metabolic functions and a zonation pattern. However, limited knowledge exists regarding zone-specific lipid metabolism in the liver in MASH. To investigate lipid metabolism across liver zonation in a MASH mouse model, we developed and deployed a spatial lipidomics approach using desorption electrospray ionisation mass spectrometry imaging (DESI-MSI).

Method: Frozen liver sections from the diet- and chemical-induced MASH models (n = 5) and controls (n = 5) were subjected to DESI-MSI analysis. Imaging was performed at a pixel size of 50 x 50 µm and a DESI stage speed of 200 µm/s. Regions of interest (ROIs) were manually delineated using histological data to determine the spatial distribution of hepatic lipids across liver zones. Mass lists of specific ROIs were generated automatically, and univariate and multivariate data analyses were conducted to examine zone-specific lipid alterations.

Results: We identified 130 lipids from various lipid classes that exhibited significant alterations across liver zones compared to the control. Fatty acids (FAs), phospholipids, diacylglycerols (DAGs), triacylglycerols (TAGs), ceramides (Cers), and sphingolipids (SLs) displayed zone-specific alterations in MASH livers compared to controls. Notably, lipid species such as TAGs, DAGs, SLs, and Cers, which were localised predominantly in the pericentral zone in the control group, extended to the periportal area in MASH. Increased hepatic lipids in the MASH group were primarily composed of saturated fatty acids and monounsaturated fatty acids, while reduced hepatic lipids contained polyunsaturated fatty acids as the major fatty acid composition. Furthermore, zone-specific lipid signatures were altered in MASH livers. In addition, fibrosis was significantly higher in the periportal region, which may contribute to the observed metabolic alteration across liver zonation.

Conclusion: Our study provides novel insights into zone-specific hepatic lipid metabolism in MASH. The disturbance and perturbation of spatial lipid metabolism appear to be implicated in the development of MASH. Understanding zone-specific lipid metabolism may enhance our understanding of MASH pathogenesis and potentially facilitate the identification of diagnostic markers associated with liver zonation.

Figure:


Oil red O
Strius red
H&E
Ballooning
Mallory-Donk bodies
Necroinflamatory foci
Macro-, micro- steatosis
Control
MASH
Tissue sectioning
DESI
Liver section
Red and blue overlay ion image from DESI-MSI
Immunofluorescence-stained liver section
CV
PT
PI 18:0_20:4 Pericentral
PE 16:0_22:6 Periportal
GS-6: Pericentral hepatocytes
E-cad: Periportal hepatocytes
DAPI: Nucleus

OS-6-YI

Higher hepatic glucose production and gluconeogenesis are features of metabolic dysfunction-associated steatohepatitis with advanced liver fibrosis, even in the absence of diabetes: insights from stable-isotope flux analysis and genome-scale study

Silvia Sabatini[1], Partho Sen[2 3], Fabrizia Carli[1], Samantha Pezzica[1], Chiara Rosso[4], Erminia Lembo[5],Ornella Verrastro[5], Ann K Daly[6], Olivier Govaere[6 7], Simon Cockell[6], Tuulia Hyötyläinen[8], Geltrude Mingrone[5 9], Elisabetta Bugianesi[4], Quentin Anstee[6], Matej Orešič[2 10], Amalia Gastaldelli[1]
[1]Institute of Clinical Physiology, CNR, Cardiometabolic Risk Unit, Pisa, Italy, *[2]University of Turku and Åbo Akademi University, Turku Bioscience Centre, Turku, Finland*, *[3]Technische Universität München, Deutsches Herzzentrum München, Munich, Germany*, *[4]University of Turin, Department of Medical Sciences, Turin, Italy*, *[5]Università Cattolica del Sacro Cuore, and Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Department of Medical and Surgical Sciences, Rome, Italy*, *[6]Newcastle University, Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle upon Tyne, United Kingdom*, *[7]KU Leuven and University Hospitals Leuven, Department of Imaging and Pathology, Leuven, Belgium*, *[8]Örebro University, School of Science and Technology, Örebro, Sweden*, *[9]King's College London, Division of Diabetes and Nutritional Sciences, School of Cardiovascular and Metabolic Medicine and Sciences, London, United Kingdom*, *[10]Örebro University, School of Medical Sciences, Örebro, Sweden*
Email: silvia.sabatini2@student.unisi.it

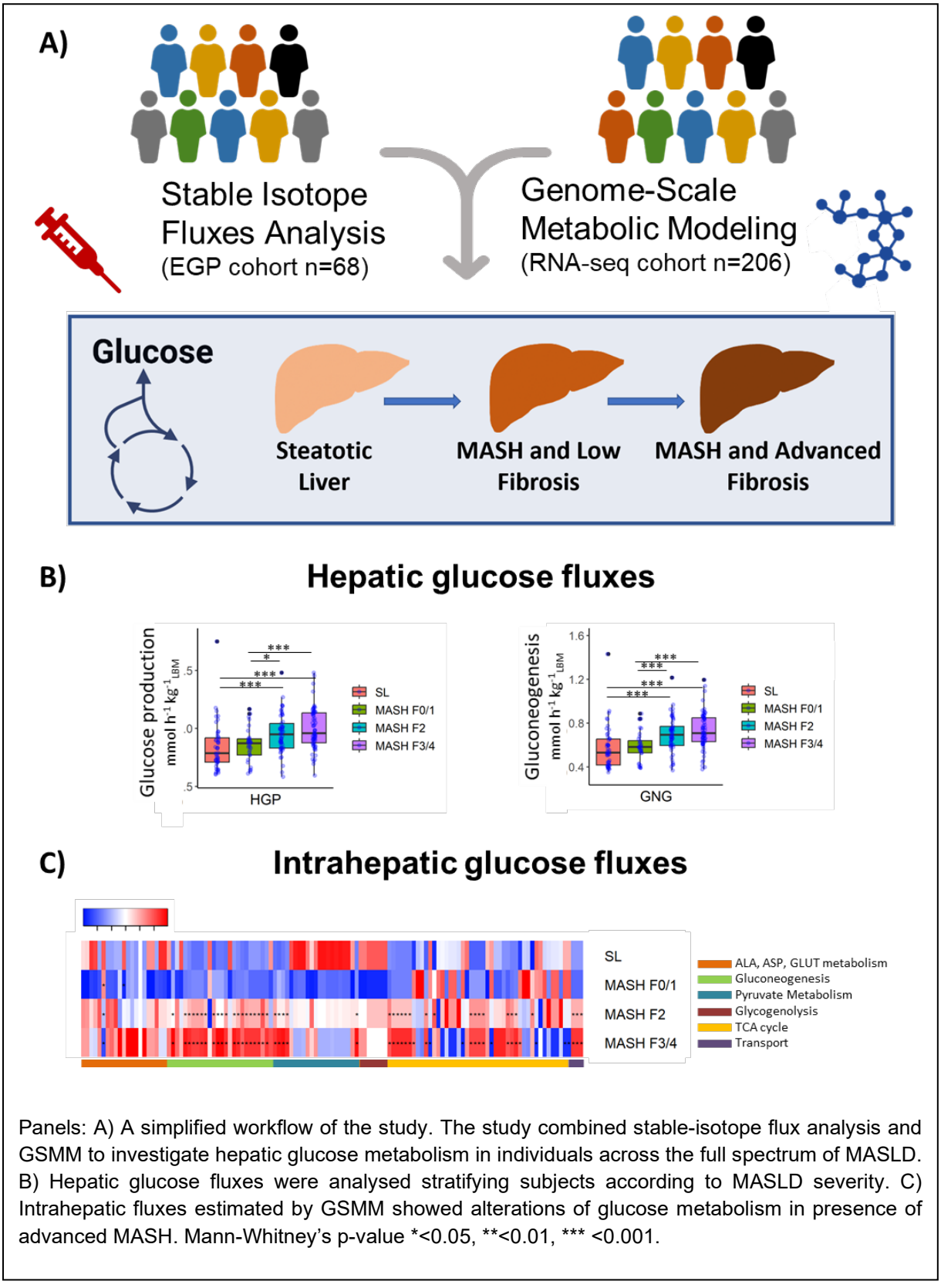
Background and aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) and its progressive form, steatohepatitis (MASH), are associated with a higher prevalence of type 2 diabetes (T2D) and obesity. In addition to its impact on lipid metabolism, individuals with MASLD commonly exhibit hepatic insulin resistance and altered glucose metabolism. The aim of this study was to investigate hepatic glucose fluxes and the molecular mechanisms that control hepatic glucose metabolism within the full spectrum of MASLD in humans.

Method: Fasting endogenous glucose production (EGP) and hepatic insulin resistance were quantified by stable-isotope (6, 6-2H2-glucose) infusion in subjects with biopsy-proven steatotic liver disease (n = 68). Moreover, intra-hepatic glucose fluxes were estimated using genome-scale metabolic modeling (GSMM) of liver transcriptomic data from the European NAFLD Registry (n = 206). Then, a separate cohort with in vivo measurement of gluconeogenesis and EGP, by $^{2}H_2O$ and 6, 6-$^{2}H_2$-glucose (n = 48), was used to validate GSMM.

Results: Tracer-measured EGP and hepatic insulin resistance were increased with MASH severity. GSMM showed that such increase was due to the upregulation of gluconeogenesis, anaplerosis and increased uptake of glucose precursors, mainly lactate and glycerol rather than upregulation of glucogenic gene expression. On the contrary, critical genes involved in insulin signaling (IRS1, IR2, and AKT2) showed reduced expression as the severity of MASH increased. The observed alterations in hepatic glucose metabolism were associated mainly with the severity of liver fibrosis but not with steatosis and were more pronounced in subjects with obesity and T2D.

Conclusion: This novel approach that combines stable-isotope tracer fluxomics and GSMM showed the importance of investigating hepatic glucose metabolism in MASLD and explains, at least in part, the mechanisms for increased risk of diabetes in subjects with advanced steatotic liver disease. Future intervention aiming to decrease the availability of glucogenic substrates and improve insulin action should be also considered in the treatment of MASLD/MASH even in absence of diabetes.

Figure:



Panels: A) A simplified workflow of the study. The study combined stable-isotope flux analysis and GSMM to investigate hepatic glucose metabolism in individuals across the full spectrum of MASLD. B) Hepatic glucose fluxes were analysed stratifying subjects according to MASLD severity. C) Intrahepatic fluxes estimated by GSMM showed alterations of glucose metabolism in presence of advanced MASH. Mann-Whitney's p-value *<0.05, **<0.01, *** <0.001.

POSTER ABSTRACT PRESENTATIONS

BASIC SCIENCE

PO1-01
DMF-activated Nrf2 ameliorates palmitic acid toxicity in hepatocytes while potentiates ferroptosis: independent protection on NO-donors

Diana Abu halaka[1], Adi Shpaizer[1], Oren Tirosh[1], Haim Zeigerman[1]
[1]*Institute of biochemistry, school of nutritional sciences, food science and nutrition, the Robert h. Smith faculty of agriculture, food and environment, the Hebrew university of Jerusalem, Rehovot, Israel*
Email: Diana.abuhalaka@mail.huji.ac.il

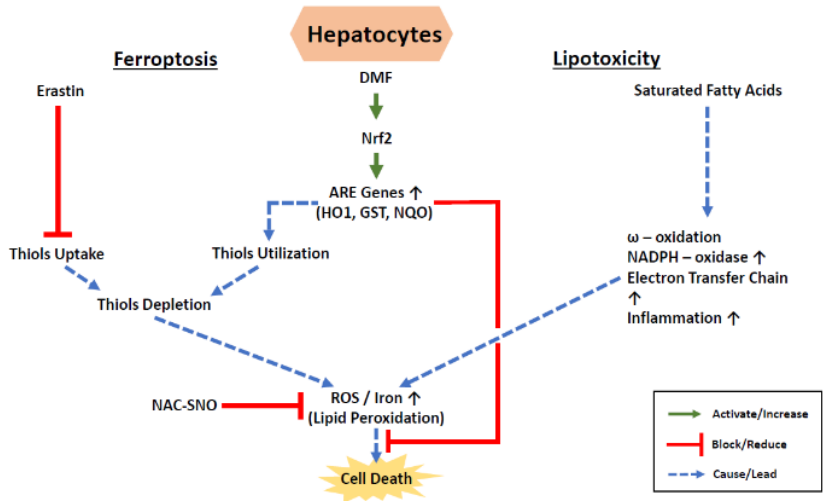
Background and aims: Non-alcoholic fatty liver disease (NAFLD) is an increasingly important global public health problem comprises a spectrum of hepatic pathology, range from simple steatosis (SS) to non-alcoholic steatohepatitis (NASH) and cirrhosis. Currently, the deterioration of NAFLD is attributed to the accumulation of free fatty acids in hepatocytes and their adaptation to toxic lipids and their byproducts which leads to mitochondrial dysfunction, inflammation, oxidative stress, and ultimately cell death-a phenomenon called lipotoxicity. Ferroptosis, an iron-dependent cell death associated with elevated lipid peroxidation, is also involved in NAFLD deterioration. In the current research, we aimed to elucidate the involvement of the antioxidant defense system of the nuclear factor erythroid-derived-2-like 2 (Nrf2)-Kelch-like ECH-associated protein 1 (Keap1)-antioxidant response element (ARE) pathway by the Nrf2 activator dimethyl fumarate (DMF) in ferroptosis and lipotoxicity. In addition, the role of different nitric-oxide (NO) donors in inhibiting ferroptosis was evaluated.

Method: To induce lipotixicity, AML12 hepatocytes were exposed to 600 µM palmitic acid (PA). Hepatocytes were treated with DMF 6 hours prior to PA treatment or simultaneously for total 24 hours. Ferroptosis was induced by 20 µM of erastin, an inhibitor of the cystine-glutamate antiporter system Xc− or 5 µM RSL3, an inhibitor of glutathione peroxidase 4 (GPX4) with the different NO-donors for 24 hours. Cell viability, oxidative stress, and the expression of Nrf2 protein and related genes were evaluated.

Results: Pre-treatment with DMF in hepatocytes was shown to protect against PA-induced lipotoxicity. This pre-treatment was effective in the activation of Nrf2-signaling pathway and elevation of dependent genes expression. On the other hand, under conditions of ferroptosis, DMF exacerbated cell death due to depletion of the intracellular thiol pool but the NO-donor, S-nitroso-N-acetylcysteine (NAC-SNO) proved to be effective in the ameliorating of hepatocytes ferroptosis with no inhibition of Nrf2. Also, the levels of lipid peroxidation were generally reduced when DMF pre-treatment is followed by exposure to palmitic acid. Conversely, DMF significantly increased lipid peroxidation when combined with erastin and NAC-SNO was able to decrease it to control levels.

Conclusion: The current study elucidates the dual role of Nrf2 in hepatocyte cell death and provides new insights into the pathophysiology of lipid toxicity. This study also demonstrated the involvement of NO as a regulator of cell death in hepatocytes and as an effective inhibitor of ferroptosis but with less involvement in lipotoxicity.

Figure:



PO1-08
Galectin-3 inhibitor prevents metabolic dysfunction-association steatohepatitis and insulin resistance in western diet-fed ApoE knockout mice

Layanne Araujo[1], Felipe Sucupira[1], Carolina Dias[1], Andressa Amaral[2], João Paulo Camporez[1]
[1]University of São Paulo, Department of Physiology, Ribeirão Preto, Brazil, *[2]University of São Paulo, Department of Biophysics and Physiology, São Paulo, Brazil*
Email: lay_biomed@yahoo.com.br

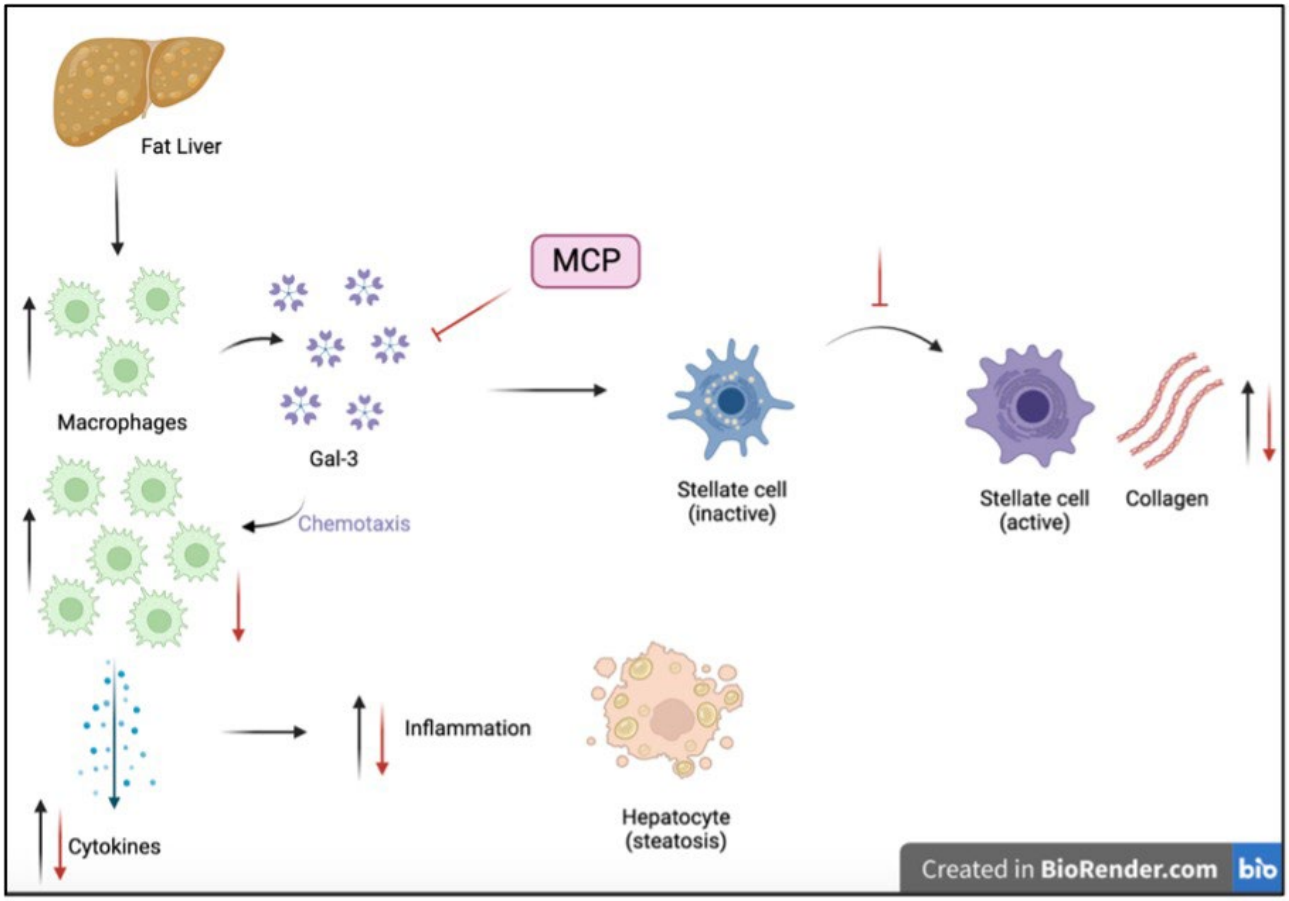
Background and aims: metabolic dysfunction-association steatohepatitis (MASH) is characterized by steatosis (accumulation of lipids in more than 5% of hepatocytes), inflammatory infiltrate and hepatocellular ballooning, and it can present fibrosis and Mallory's bodies. The aim of this study is to evaluate the protective potential of the Gal-3 inhibitor (MCP-modified citrus pectin) on MASH and insulin resistance.

Method: ApoE KO mice were divided into the following groups: standard diet fed (SD), western diet fed (WD) and western diet + fructose fed and MCP treated (WD + MCP). MCP was administered orally in drinking water (1%). The animals were fed with WD for 7 weeks, after the 3rd week of diet, the treatment with MCP was started, and lasted 4 weeks.

Results: WD promoted an increase in the area under the curve (AUC) of glucose tolerance test (339.5 ± 19.7 mg/dL-min) and glycemia (191.4 ± 8.2 mg/dL), plasma insulin (1.2 ± 0.3 ng/ml) and HOMA-IR (10.0 ± 1.9), when compared to the SD group. While MCP treatment inhibited the increase of all these parameters. WD increased liver TAG accumulation by 22% and plasma TAG (68.0 ± 2.6 mg/dL), cholesterol (318.0 ± 21.6 mg/dL), fatty acids (0.2 ± 0.04 mEq/L) and VLDL (13.6 ± 0.5 mg/dL). However, MCP treatment was able to inhibit this increase of TAG in the tissue by 9% and in the plasma (41.0 ± 2.7 mg/dL), and VLDL (8.2 ± 0.5 mg/dL), however had no significant difference for cholesterol and fatty acids in relation to WD group. We analyzed the expression of genes involved in the lipogenesis pathway in the liver, such as SREBP1, ACC, SCD1 and FASn. Only ACC gene expression was increased by 10x and SREBP1 protein expression by 183% in the WD group compared to the SD group. And the MCP treatment inhibited the increase of both. The animals of WD group showed a greater deposition of collagen in the liver (18%), as well as the increase of genes expression related to collagen production (TGF-Beta = 4-fold, Col1alpha = 3-fold, MMP2 = 5-fold), compared to SD group. While MCP treatment inhibited the increase in collagen deposition by 8.2% and only MMP2 gene expression. In addition, the galectin-3 protein expression was analyzed, and it was increased by 500% in the WD group compared to the SD group, and this increase was inhibited by 200% with MCP treatment. The inflammatory profile of the liver was also shown, obtaining a 3-fold increase in the expression of F4/80 (marker of macrophage activity), gene expression of pro-inflammatory cytokines (TNFalpha = 4-fold and IL-1Beta = 3-fold) and protein activity of pro-inflammatory pathways (pJNK) by 166% in the WD group compared to the SD group. Meanwhile, MCP treatment inhibited the increase of all these parameters.

Conclusion: Given these results, it is concluded that the galectin-3 inhibitor (MCP) protects the liver from the progression of steatohepatitis in animals fed western diet and fructose.

Figure: The black arrows correspond to the process promoted by steatohepatitis, while the red arrows indicate the processes that occur after of the effect of MCP



PO1-09
Pharmacodynamics screening of potential therapeutic substances in an experimental model of metabolic dysfunction associated steatohepatitis

Mahak Arora[1], Tijana Šopin[2], Tomáš Vacík[2], Zuzana Pavlíková[3 4], Tomáš Kučera[3], Nikolina Kutinová Canová[1]
[1]Institute of Pharmacology, First Faculty of Medicine, Charles University and General University Hospital, Prague 2, Czech Republic, *[2]Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University and General University Hospital, Prague 2, Czech Republic*, *[3]Institute of Histology and Embryology, First Faculty of Medicine, Charles University, Prague 2, Czech Republic*, *[4]Department of Anthropology and Human Genetics, Faculty of Science, Charles University, Prague 2, Czech Republic*
Email: Mahak.Arora@lf1.cuni.cz

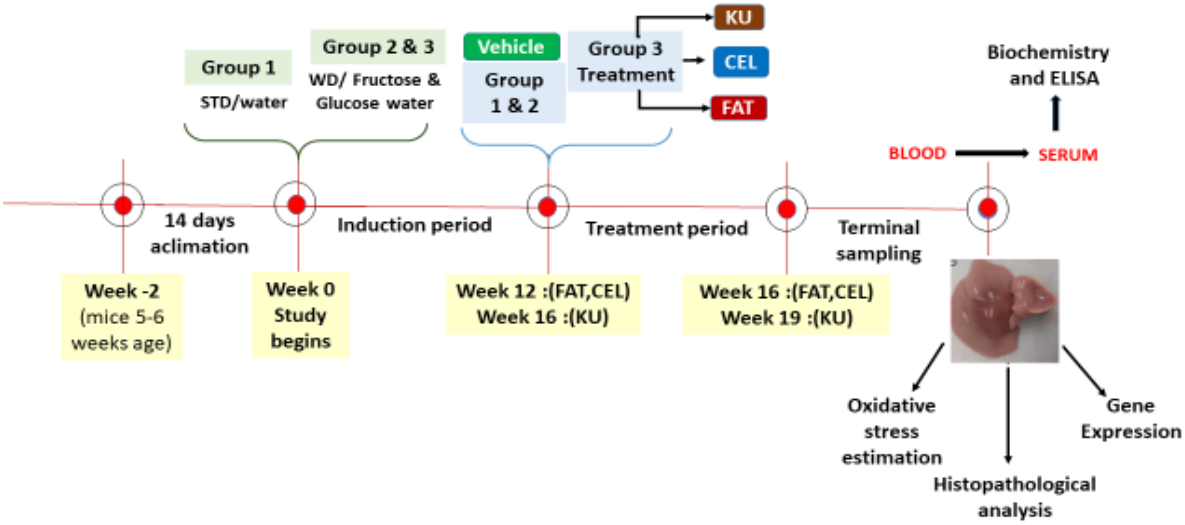
Background and aims: Metabolic dysfunction-associated steatohepatitis (MASH) if untreated leads to liver fibrosis, cirrhosis and cancer. As there is no specific pharmacological MASH treatment, we screened some candidate drugs. We focused on drugs modulating metabolic molecular targets affecting lipogenesis and adipogenesis like non-specific mTORC1/C2 (mammalian target of rapamycin) inhibitor Ku-0063794 (KU), a sterol regulatory element-binding protein (SREB) inhibitor fatostatin (FAT), and a galanin receptor inhibitor celastrol (CEL). The aim of the study was to investigate the potential therapeutic effects of KU, FAT and CEL in dietary model of MASH progression in mice.

Method: Male C57BL6J mice were randomized into 3 groups with each drug being tested separately. For induction, always group 1 received the standard chow diet (STD) with water while mice in group 2 and 3 were fed by special atherogenic western diet (WD) with fructose and glucose in drinking water for 12 or 16 weeks marking MASH onset and progression, respectively. It was followed by the intraperitoneal treatment for 3-4 weeks along with the diet. Groups 1 and 2 received vehicle and group 3 was treated by the specified drug: KU (5 mg/kg) daily for 3 weeks, FAT (15 mg/kg) each 2nd-3rd day and CEL (200 microg/kg) each 2nd day for 4 weeks. Then terminal samplings of blood and livers were done for further analysis (Schematic figure).

Results: The treatment with KU was safe and significantly improved some features of MASH hepatotoxicity like liver oxidative stress markers, triglycerides, TNF-alpha mRNA levels and mitochondrial ATP production. FAT and CEL significantly reduced glycaemia, body, fat and liver weights, liver enzymes, total cholesterol, liver triglycerides, steatosis and histopathological total NAFLD activity score. FAT, however, produced skin toxicity (resembling eczema) and systemic inflammation (TNF-alpha) while CEL was safe and remarkably decreased expression of proinflammatory and lipogenic genes.

Conclusion: Although the tested agents hit different molecular targets, all were beneficial to some extent in reducing MASH severity. KU was least effective, but unlike other selective mTOR inhibitors (e.g. rapamycin), it was safe. FAT corrected metabolic abnormalities and slowed the histopathological progression of MASH, however, it induced skin toxicity. Since CEL demonstrated high efficacy and safety, it emerges as an efficient candidate for further testing. This work was supported by GAUK 190/54/251753, SVV 260523, and Cooperatio_PHAR.

Figure:


Group 1
STD/water
Group 2 & 3
WD/ Fructose & Glucose water
Vehicle
Group 1 & 2
Group 3 Treatment
KU
CEL
FAT
Biochemistry and ELISA
BLOOD
SERUM
14 days aclimation
Induction period
Treatment period
Terminal sampling
Week -2 (mice 5-6 weeks age)
Week 0 Study begins
Week 12 :(FAT,CEL) Week 16 :(KU)
Week 16 :(FAT,CEL) Week 19 :(KU)
Oxidative stress estimation
Histopathological analysis
Gene Expression

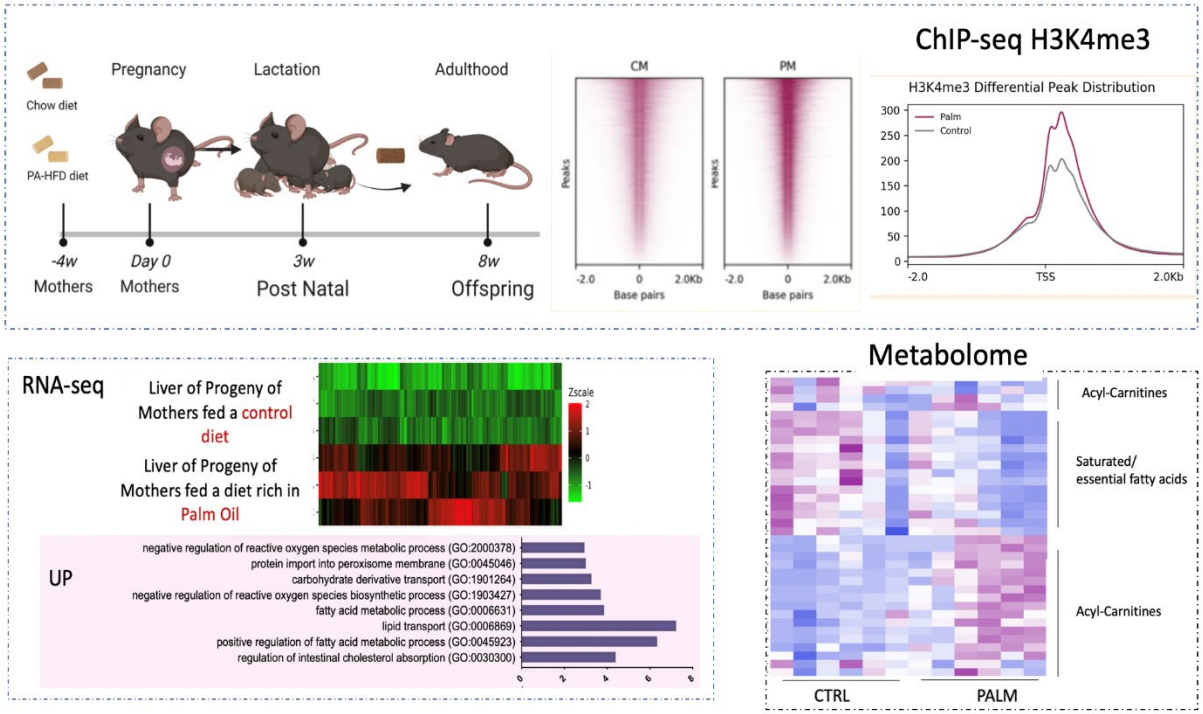
PO1-12
Maternal overnutrition and increased risk of intergenerational liver disease and cancer-targeting epigenetic mechanisms

Salvador Aznar-Benitah[1 2], Claudia Bigas[2], Blanca Majem[2], Josefina Martin[2], Gloria Pascual[2]
[1]*ICREA*, [2]*IRB Barcelona, Stem Cells and Cancer Group, Barcelona, Spain*
Email: claudia.bigas@irbbarcelona.org

Background and aims: Obesity and malnutrition have become widespread issues, contributing to the development of metabolic disorders and cancers. Maternal obesity and nutrition have been identified as strong predictors of adverse health outcomes in offspring, including fatty liver disease and liver cancer. Recent studies have shown that chronic maternal exposure to a high-fat diet during pregnancy leads to increased susceptibility to liver cancer and fatty liver disease in offspring (Sun et al., 2020; Wesolowski et al., 2017). This observation should be underscored, since it indicates that high consumption of saturated fatty acids is a risk factor by itself towards intergenerational liver cancer propensity. What's more, these alterations persist into the adult stage, despite feeding the offspring a healthy diet after weaning. These findings emphasize that exposure to specific nutrient conditions during pregnancy and lactation are critical windows for NAFLD and liver cancer predisposition of the offspring in later life.However, the mechanism behind this intergenerational transmission of liver cancer predisposition is not yet understood. Notably, data from our lab (Pascual et al. (2021))shows that palmitic acid, one of the most consumed saturated fatty acids in Western diets, has been found to have long-term pro-metastatic effects in tumors through epigenetic mechanisms. This study aims to investigate whether maternal intake of a palmitate-based HFD during pregnancy and lactation predisposes offspring to liver disease and cancer, and if this intergenerational propensity for liver cancer is due to epigenetic changes of the offspring's epigenome in the liver. Additionally, the study aims to investigate whether these epigenetic changes are due to stable deposition of H3K4me3 downstream of the Set1a signaling axis in the liver.

Results: Maternal consumption of a high fat diet based on palm oil epigenetically alters lipid metabolism-related genes in the liver of F1 offspring. We have verified whether our observations in metastatic cells corroborate the hypothesis of this grant proposal regarding the intergenerational transmission of epigenetic alterations to the livers of the progeny of F1 offspring exposed to high fat diets during pregnancy and lactation. Strikingly, our unpublished results show that the livers of the offspring of mothers fed with a palm oil enriched-HFD show stable changes in H3K4me3 at their promoters, and stable transcriptome changes at 3 weeks of age that persist at 8 weeks of age, and are related to aberrant fatty acid metabolism, oxidation, and inflammation (Fig.1). Importantly, whole metabolome/lipidome studies shows that these epigenetic and transcriptomic changes are accompanied by the aberrant accumulation of acyl-carnitines in the liver of these mice, which indicates heightened fatty acid oxidation (Fig.1).We believe this will be the first time that an epigenetic mechanism is identified as responsible for an increased risk of liver disease and cancer in offspring due to parental overnutrition, which would make it of high-interest as a disease biomarker and for the development of new therapies.

Figure: Epigenetic, transcriptomic and metabolic memory in the liver of PO DAM fed F1 offspring.



PO1-13-YI
Inflammatory landscape and metabolic profiling of the Mc4r KO mouse on a western diet to study human metabolic dysfunction-associated steatohepatitis

Fabienne Birrer[1 2], Tess Brodie[1 2], Tural Yarahmadov[1 2], Mojgan Masoodi[3], Deborah Stroka[1 2]
[1]Department of Visceral Surgery and Medicine, Inselspital, Bern University Hospital, University of Bern, Switzerland, *[2]Department for BioMedical Research, Visceral Surgery and Medicine, University of Bern, Switzerland*, *[3]Institute of Clinical Chemistry, Inselspital, Bern University Hospital, Bern, Switzerland*
Email: fabienne.birrer@unibe.ch

Background and aims: Metabolic dysfunction-associated steatohepatitis (MASH) is a progressive form of metabolic dysfunction-associated steatotic liver disease (MASLD) characterized by inflammation, liver cell injury and fibrosis. Animal models play a crucial role in studying the underlying disease mechanisms. However, often it is questioned if they reflect the whole spectrum of complexity observed in human disease. The melanocortin-4-receptor knock out (Mc4r KO) mouse fed a Western type diet (WD) is already described to be similar to human MASH, but characterization is not yet complete. In this project we aimed to describe a specific inflammatory landscape and metabolic profile in Mc4r KO MASH mice using imaging mass cytometry (IMC) and bulk RNA sequencing (RNAseq).

Method: Eight-weeks old, male Mc4r KO mice were fed either normal chow or WD for 25 weeks. Three experimental groups were set up: WT on normal chow (control), Mc4r KO on normal chow (MASLD) and Mc4r KO on WD (MASH). Liver tissues were categorized into MASLD and MASH using hematoxylin/eosin (HE) and sirius red staining to assess steatosis, inflammation, hepatocyte ballooning and fibrosis. For IMC, formalin fixed and paraffin embedded tissues were stained with a 29-target panel to detect liver parenchymal and immune cells. Bulk RNA-seq data was collected from the livers of all three conditions for a comprehensive metabolic pathway analysis and the altered metabolic pathways were compared to human MASH.

Results: The HE and sirius red staining showed massive steatosis (macro- and microvesicular, >80%), inflammation (grade 1), hepatocyte ballooning (grade 2) and fibrosis (F1/F2) in the MASH livers, whereas MASLD only showed steatosis (30-80%). Control livers did not show any signs of the assessed parameters. Examining structural markers such as E-cadherin in the IMC images, we found a compromised structural integrity in the MASLD livers, which was persistent and even more profound in the MASH condition. After cell segmentation and population annotation, variations in cell numbers and cell distribution within the tissue were visualized and differences in the distribution patterns of specific populations could be detected. MASH mouse livers showed specific cell communities that clustered predominantly within this condition, but not in control or MASLD livers. Populations that were found inside these communities tended to be mainly macrophages and T-cells.

Conclusion: We successfully implemented the Mc4r KO MASH model in our facility and generated control, MASLD and MASH mice. Our study describes the changes in the inflammatory landscape from control to MASH livers in our Mc4r-KO mouse model and identifies specific cell communities unique to MASH. These communities could support the identification of novel targets in inflammation and disease progression. In addition, we evaluated the altered metabolic pathways and metabolic signature in our mouse model, which can further facilitate translational research in this specific model.

PO1-15
STE20-type Kinases MST3 and MST4 promote the progression of hepatocellular carcinoma

Mara Caputo[1]
[1]*University of Gothenburg, Department of Chemistry and Molecular Biology, Gothenburg, Sweden*
Email: mara.caputo@gu.se

Background and aims: Hepatocellular carcinoma (HCC) is one of the most fatal and fastest-growing malignancies. Recently, non-alcoholic steatohepatitis (NASH), characterized by liver steatosis, inflammation, cell injury (hepatocyte ballooning), and different stages of fibrosis, has emerged as a major catalyst for HCC. Because the STE20-type kinases MST3 and MST4 have been described as critical molecular regulators of NASH pathophysiology, we focused on determining these proteins' relevance in human HCC.

Method: The clinical importance of MST3 and MST4 in HCC was assessed in publicly available datasets and by qRT-PCR analysis of a validation cohort recruited at the University Hospital of Tübingen (n = 48 for HCC patients and n = 214 for control subjects). The functional significance of MST3 and MST4 was examined in HepG2 and Hep3B cells transfected with *MST3*, *MST4*, or *MST3/4* small interfering RNA. Potential downstream pathways were investigated by co-immunoprecipitation and Western blotting.

Results: By analyzing public datasets and in-house cohorts, we found that hepatic *MST3* and *MST4* expression was positively correlated with the incidence and severity of HCC. We also found that the silencing of both MST3 and MST4, but also either of them individually, markedly suppressed the tumorigenesis of human HCC cells including attenuated proliferation, migration, invasion, and epithelial-mesenchymal transition. Mechanistic investigations revealed lower activation of STAT3 signaling in MST3/MST4-deficient hepatocytes and identified GOLGA2 and STRIPAK complex as the binding partners of both MST3 and MST4 in HCC cells.

Conclusion: These findings reveal that MST3 and MST4 play a critical role in promoting the progression of HCC and suggest that targeting these kinases may provide a novel strategy for the treatment of liver cancer.

Figure:

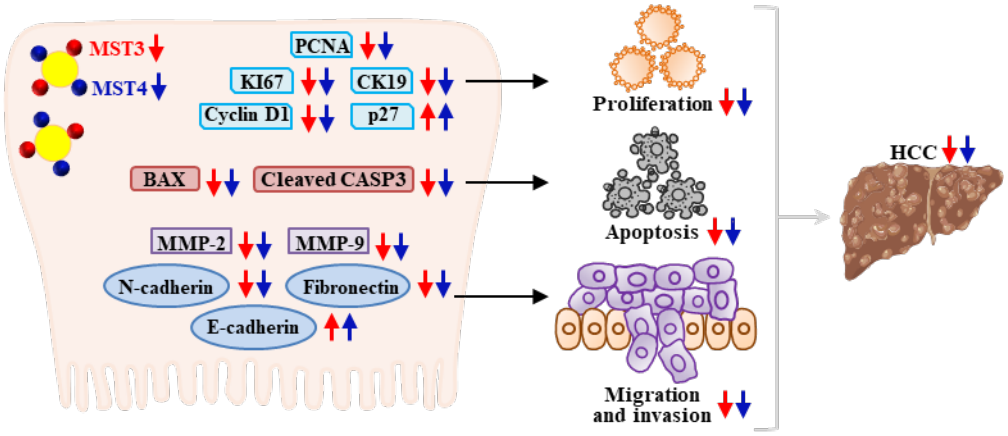


Figure1. *Silencing of MST3 and/or MST4 protects against HCC development and progression by alleviating proliferation and apoptosis and by suppressing the migration, invasion, and EMT of human HCC cells.*

PO1-16-YI
RIPK3-dependent signalling and crosstalk hepatocytes-macrophages in MASLD progression

André F. L. Cardador[1], Marta B. Afonso[1], Mariana Alves[1], Ana C. Pêgo[2], Maria Manuela Gaspar[1], Raffaella Gozzelino[2], Jérémie Gautheron[3 4], Cecilia Rodrigues[1]
[1]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal, *[2]Chronic Diseases Research Center (CEDOC), Nova Medical School (NMS), NOVA University of Lisbon, Lisbon, Portugal*, *[3]Institute of Cardiometabolism and Nutrition (ICAN), Paris, France*, *[4]Sorbonne Université, Inserm, Centre de Recherche Saint-Antoine (CRSA), Paris, France*
Email: aflcardador@gmail.com

Background and aims: The receptor-interacting protein kinase 3 (RIPK3) is critical in two different lytic, inflammatory types of regulated cell death activated in metabolic dysfunction-associated steatotic liver disease (MASLD), namely necroptosis and inflammasome-mediated pyroptosis. Still, the cell-specific role of RIPK3 in MASLD remains unclear. Here, we aim to characterize the role of RIPK3 in the crosstalk between hepatocytes and macrophages in the pathogenesis and progression of MASLD.

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 (CD-HFD) or a standard diet (SD) from 4 to 42-weeks-old. In parallel, mice were fed from 4 to 57-weeks-old with CD-HFD. Gene expression analyses were performed to evaluate markers of inflammation and infiltrated immune cells. Further, wild-type (WT) and CRISPR-Cas9 *Ripk3*-null (*Ripk3*$^{-/-}$) AML12 murine hepatocytes were incubated with 125 µM palmitic acid (PA) conjugated with bovine albumin serum or vehicle control for 24 h, followed by co-culture with J774A.1 murine macrophages from physiological 8:1 and 4:1 ratios to inflammation 2:1 ratio without further treatment.

Results: *Ripk3* deficiency reduced hepatic inflammatory response in both CD-HFD-fed and DEN-treated mice. Importantly, macroscopically discernible tumours were only detected in mice treated with DEN and DEN+CD-HFD, while tumour burden was significantly reduced by *Ripk3* deficiency. Further, *Pd-l1* and *Pd-1* were globally reduced in *Ripk3*$^{-/-}$ mice, compared with WT counterparts, except in tumours from DEN+CDHFD. This was accompanied by a general decrease in expression of *Nlrp3* and its downstream effectors in pyroptosis, caspase-1 and *Il1β*. In line, *Ifnγ*, the main regulator of *Pd-l1*, was generally reduced in *Ripk3*$^{-/-}$ mice. *In vitro*, the expression of the pro-inflammatory markers *Tnf-α*, *Il1β*, *Inos, Nlrp3*, and *Cd86* was exclusively increased in co-cultures of PA-treated WT hepatocytes with macrophages at the inflammation 2:1 ratio. In turn, macrophages in co-culture with PA-treated *Ripk3*$^{-/-}$ hepatocytes displayed a less inflammatory profile.

Conclusion: Our results show that *Ripk3* deficiency ameliorated hepatic inflammation in preneoplastic experimental MASLD, while reducing the hepatic tumour burden *in vivo*. Further, *Ripk3* deletion might impact on PD-L1/PD-1 axis, likely by impairing NLRP3 inflammasome activation, which might impact on disease progression and treatment response. Finally, blocking RIPK3-dependent signaling in hepatocytes may hinder inflammation upon free fatty acids overload, possibly by inhibiting hepatocyte immunogenic cell death. (Supported by 2021.07666.bd and PTDC/MED-FAR/3492/2021, FCT; LCF/PR/HR21/52410028, La Caixa Foundation)

PO1-17
SAMM50 affect mitochondria dynamics in high-fat related non-alcohol steatohepatitis

Wan-Long Chuang[1], Shu-Chi Wang[2], Jee-Fu Huang[1], Ming-Lung Yu[1]
[1]Kaohsiung Medical University, Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, *[2]Kaohsiung Medical University, Department of Medical Laboratory Science and Biotechnology, Kaohsiung, Taiwan*
Email: shuchiwang@gmail.com

Background and aims: Non-alcoholic steatohepatitis (NASH), an extreme form of non-alcoholic fatty liver disease (NAFLD), may lead to liver-related complications such as fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). Mitochondrial dynamics, which govern energy balance and mitochondrial function, are engaged into the pathogenic processes. Sorting and Assembly Machinery Component 50 homolog (SAMM50) is an outer mitochondrial membrane protein, and plays a pivotal role in regulating mitochondrial dynamics.

Method: In this study, we established an NAFLD/NASH animal model using a high-fat Western diet and high-fructose drinking for 7 months. AML-12 hepatocytes were cocultured with 3T3-L1 adipocytes conditioned medium (Adipo-CM) to stimulate pre-injury hepatic steatosis. H_2O_2 was treated (Adipo-CM+H_2O_2) aiming to mimic significant hepatic steatosis with oxidative stress environment. We also used shRNA to knockdown SAMM50 expression in order to explore the changes in mitochondrial function and morphology.

Results: The HFSD-induced NAFLD/NASH animal model showed differential effects on SAMM50 regulation and expression in the early and late stages of hepatic steatosis initiation. The significant increases of ATP synthesis, mtDNA, or mitochondria ROS were observed in Adipo-CM+H_2O_2, but not in Adipo-CM cell model. Knockdown of SAMM50 by shSAMM50 showed the significant decrease of mitochondrial biogenesis protein (PGC-1α) and mitochondrial dynamics fusion/fission-associated protein (OPA1, FIS1, Drp1) expression in Adipo-CM+H_2O_2 model. Furthermore, the reduction in SAMM50 expression led to a decrease of cellular ATP synthesis and a compensatory increase in mtDNA in the early phase of Adipo-CM-induced hepatocyte steatosis injury.

Conclusion: SAMM50 exerts a protective role in hepatocyte steatosis injury by modulating the expression of mitochondrial biogenesis and fission-related proteins, thereby maintaining mitochondrial function and morphology.

PO2-02
Efficacy of metabolyteTM treatment for MASH prevention and reversal

Giulia Angelini[1], Sara Russo[1], Amalia Gastaldelli[2], Silvia Sabatini[2], Geltrude Mingrone[1]
[1]Università Cattolica del Sacro Cuore, Translational medicine and surgery, ROMA, Italy, *[2]National Research Council, Institute of Clinical Physiology, Pisa, Italy*
Email: giulia.angelini@unicatt.it

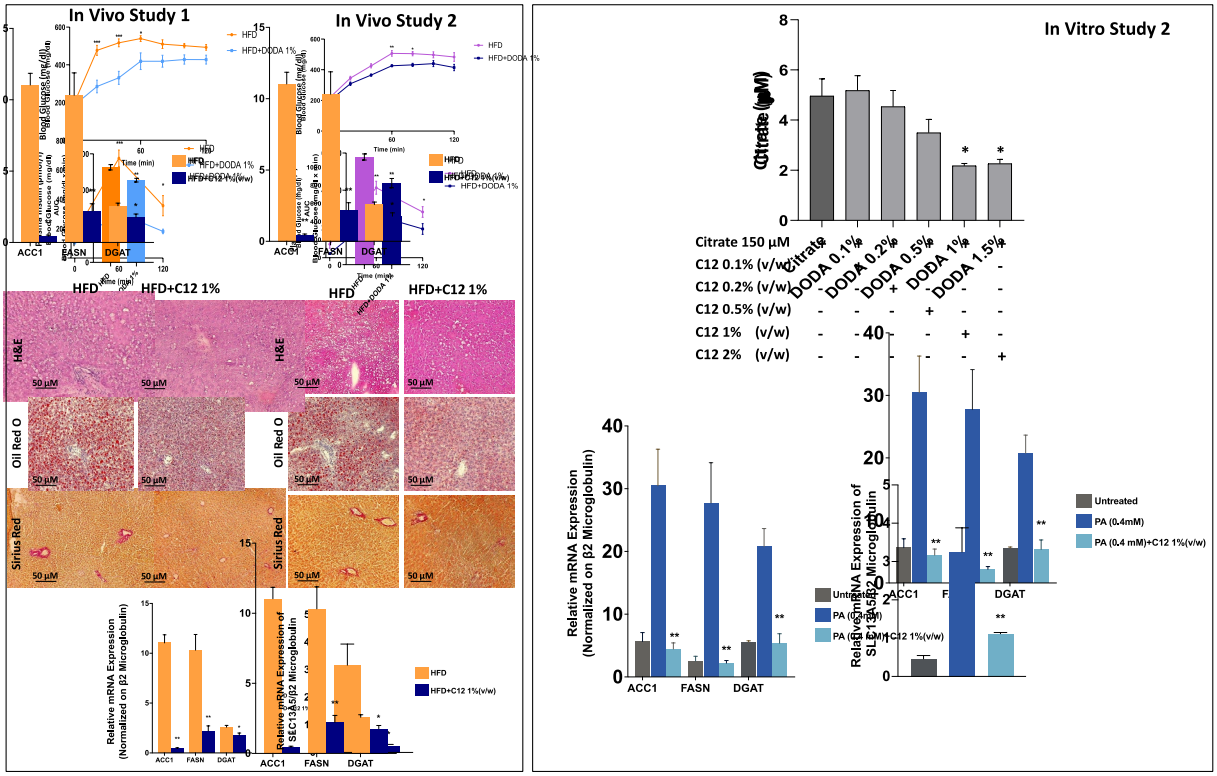
Background and aims: Metabolic steatohepatitis (MASH) has reached epidemic proportions worldwide and is associated with a twofold higher risk of developing type 2 diabetes mellitus (T2DM). The improvement of MASH is associated with a reduction of T2DM risk, suggesting that liver-focused treatments might reduce the risk of developing T2DM. Metabolyte™ (Jemyll Inc., USA) is a dodecanedioic acid (C12), which belongs to the family of straight-chain dicarboxylic acids. Following β-oxidation C12 produces succinyl-CoA that inhibits citrate synthase, a key regulator of de novo lipogenesis (DNL). The Na+-coupled dicarboxylate transporter (NaCT) mediates the transport of citrate into cells and plays important roles in determining cytosolic citrate concentrations. We hypothesized that oral administration of 1% w/vol C12 could prevent, as well as reverse, MASH by reducing citrate uptake and DNL.

Method: We performed two separate studies in rats to understand if C12 could prevent (Study-1) and/or reverse (Study-2) MASH. In Study-1, C12 was administered together with a high-fat high-cholesterol diet for 4 weeks, while in Study-2 rats were first fed the high-fat high-cholesterol diet for 9 weeks and then C12 for 4 weeks. Primary human hepatocytes were treated with C12 (0.1-0.25-0.5-1-1.5% w/vol) in the presence of citrate (150 μM) to assess citrate uptake. Stimulation of primary human hepatocytes was performed in the presence of C12 (1% w/vol) and palmitic acid (PA) (0.4mM) to assess DNL. qReal-Time PCR analysis was performed on hepatocytes and liver in both studies to assess gene expression for NaCT and DNL key enzymes. Blood glucose and plasma insulin concentrations were measured by oral glucose tolerance test.

Results: In both studies, C12 treatment reversed insulin resistance, causing a decrease in blood glucose and plasma insulin concentrations. Histological analysis revealed that C12 treatment prevented and reversed the effects of MASH. Hepatocytes treated with C12 and citrate showed a decrease in citrate uptake, while cells stimulated with C12 and palmitic acid showed a decrease of NaCT and DNL key enzymes. Concomitantly, gene expression of NaCT and DNL key enzymes was reduced in the liver of rats treated with C12.

Conclusion: This study provides evidence that oral administration of Metabolyte™ (1% w/vol) can protect against or reverse MASH reducing citrate uptake mediated by NaCT.

Figure:


In Vivo Study 1
In Vivo Study 2
HFD
HFD+DODA 1%
HFD+C12 1%(v/w)
Time (min)
ACC1
FASN
DGAT
H&E
Oil Red O
Sirius Red
50 μM
Relative mRNA Expression (Normalized on β2 Microglobulin)
In Vitro Study 2
Citrate (μM)
Citrate 150 μM
DODA 0.1%
DODA 0.2%
DODA 0.5%
DODA 1%
DODA 1.5%
C12 0.1% (v/w)
C12 0.2% (v/w)
C12 0.5% (v/w)
C12 1% (v/w)
C12 2% (v/w)
Untreated
PA (0.4mM)
PA (0.4 mM)+C12 1%(v/w)

PO2-03
RIPK3 modulates acyl chain structure in metabolic liver disease

Miguel Mateus-Pinheiro[1], Marta B. Afonso[1], André Cardador[1], Maria M. Gaspar[1], Fábio Fernandes[2], Enara Arretxe[3], Cristina Alonso[3], Jérémie Gautheron[4], Cecilia Rodrigues[1]
[1]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal, *[2]Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal*, *[3]OWL Metabolomics, Bizkaia Technology Park, Derio, Spain*, *[4]Centre de Recherche Saint-Antoine (CRSA), Institute of Cardiometabolism and Nutrition (ICAN), Sorbonne Université, INSERM, Paris, France*
Email: miguel-pinheiro@edu.ulisboa.pt

Background and aims: Metabolic dysfunction associated steatotic liver disease (MASLD) comprises a spectrum of metabolic disarrays that can precipitate the development of hepatocellular carcinoma. We identified the receptor interacting protein kinase 3 (RIPK3) as a fundamental protein in promoting metabolic dysfunction associated steatohepatitis (MASH). Here, we aimed to further elucidate the role of RIPK3 in lipid metabolism.

Method: Liver samples from C57BL/6 wild-type (WT) or *Ripk3*-deficient (*Ripk3*$^{-/-}$) mice fed choline-deficient L-amino acid-defined diet (CDAA; n = 14) or an isocaloric control choline-sufficient L-amino acid-defined diet (CSAA; n = 14) for 32 weeks were used for lipidomic analysis, followed by the gene expression analysis of relevant targets. AML12 murine hepatocytes were incubated with palmitic acid (PA) alone or mixed with oleic acid (OA) in different PA/OA ratios (POA 1/1 and 1/2) to mimic lipotoxic and steatogenic conditions, respectively. By using a fluorescent probe, membrane fluidity was evaluated in WT and Ripk3-null AML-12 cells. The toxicity of PA was addressed by analysing adenylate kinase leakage at 24 h. Intracellular lipid droplet accumulation was evaluated through Nile Red staining 48 h upon PA incubation.

Results: *Ripk3*$^{-/-}$ mice displayed less liver damage, increased body weight and increased liver fat accumulation in both MASH-inducing and control diets. *Ripk3*$^{-/-}$ mice displayed increased levels of glycerolipids with shorter acyl chains and low unsaturation in mice fed the CSAA diet, while species with longer acyl chains and high number of double bonds were decreased in *Ripk3*$^{-/-}$ comparing to WT mice. Accordingly, qPCR analysis revealed changes in proteins responsible for acyl chain saturation and elongation. Additionally, monounsaturated fatty acids were increased in *Ripk3*$^{-/-}$ mice, which was associated with an increased stearoyl-CoA desaturase-1 (SCD1) activity. Membrane fluidity was decreased in *Ripk3*$^{-/-}$ AML-12 cells, being consistent with changes in the saturation and elongation of acyl chains. Furthermore, *Ripk3*$^{-/-}$ AML-12 cells also presented an increased accumulation of intracellular lipids and less cellular death upon PA exposure.

Conclusion: In conclusion, *Ripk3* deficiency decreases lipotoxicity upon free fatty acid overload and changes the lipidome towards a beneficial profile in a dietary MASH model, namely by modulating fatty acid acyl chain structure. Thus, targeting RIPK3 might mitigate the lipid dysregulation underlying MASLD pathogenesis.

PO2-06
Will we be able to use the platelets features for non-invasive diagnostic tests or as new therapeutic targets?

Tania D'Amico[1], Salvatore Fasano[1], Ludovica Lombardi[1], Francesca Maiorca[1], Davide Pallucci[1], Ramona Marrapodi[1], Anna Maria Sabetta[1], Marzia Miglionico[1], Fabrizio Recchia[1], Nicolo' nicolo.sperduti@uniroma1.it[1], Marco Vano[1], Marcella Visentini[1], Giulio Francesco Romiti[1], Bernadette Corica[1], Roberto Cangemi[1], Adriano Pellicelli[2], Stefania Basili[1], Lucia Stefanini[1]
[1]Sapienza University, Department of Translational and Precision Medicine, Rome, Italy, *[2]San Camillo Hospital, Hepatology Unit, Department of Transplantation and General Surgery, Rome*
Email: tania.damico@uniroma1.it

Background and aims: metabolic dysfunction-associated steatotic liver disease (MASLD) is an increasingly prevalent manifestation of the metabolic syndrome with high morbidity and mortality rates. Platelets have been implicated in the progression of chronic liver disease from simple hepatic steatosis to metabolic dysfunction-associated steatohepatitis (MASH) and cirrhosis. However, the functional state of circulating platelets at various stages of liver disease is debated and poorly understood.
The aim is to identify phenotypic and functional characteristics of circulating platelets in patients MASLD and MASH. Then identify platelet characteristics that may participate in the amplification of the inflammatory state that drives disease progression.

Method: 34 patients histologically proven (n = 14 MASLD and n = 20 MASH) were enrolled. Citrated venous whole blood was processed within 1 hour of collection to characterise platelet phenotype, function and immune interactions by multi-parametric flow cytometry. The study was approved by the Ethics Committee of the Policlinico Umberto I (Rome, Italy). Written informed consent was obtained from all participants.

Results: Liver disease severity correlated with an increase of platelet size and of the immature platelet fraction, suggesting an increase in platelet turnover, even though only 20% of participants had platelet count below the normal range. Compared to MASLD, circulating platelets of MASH patients expressed higher levels of receptors implicated in immune-like functions (CD62P, CD42b, TLR4, CLEC2, CD44 and MHC-I) and exhibited a higher platelet responsiveness to agonists of Immunoreceptor Tyrosine-based Activation Motif (ITAM)-coupled receptors. However, MASLD, but not MASH patients, exhibited higher levels of platelet aggregates with CD8+T cells and NKT cells in peripheral blood, possibly because in more advanced stages of the disease these aggregates relocate to the liver.

Conclusion: The study identified platelet pro-inflammatory features that characterise early stages of chronic liver disease with utility for its non-invasive diagnosis or as novel molecular targets for its treatment. This study was funded by MERCK SHARP and DOME CORP. ClinicalTrials.gov identifier: NCT05128253

PO2-07
Transcriptomics-assisted phenotypic screening to identify novel drugs and targets for liver fibrosis

Elisabetta De Filippo[1], Maren Feist[1], Pamela Klingbeil[2], Isabel Goerke[2], Andrei Shchebet[1], Alexandra Hackmann[1], Timur Samatov[1], Ruediger Fritsch[1], Vladimir Galatenko[1], Alfiia Latfullina[1], Sebastian Kirsch[1], Susann Santag[1], Jeremie Boucher[1], Uwe Andag[1]
[1]Evotec International GmbH, Göttingen, Germany, *[2]Evotec SE, Hamburg, Germany*
Email: elisabetta.defilippo@evotec.com

Background and aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a complex and multifactorial disease, that ranges from fatty liver, characterized by increased lipid storage in hepatocytes, to the more advanced metabolic dysfunction-associated steatohepatitis (MASH) with inflammation, hepatocyte ballooning and different degrees of fibrosis. To date, chronic liver diseases accompanied with liver fibrosis have caused significant morbidity and mortality in the world with increasing tendency and no drugs are currently approved for the treatment of liver fibrosis. In order to identify novel anti-fibrotic targets and drugs, we aimed to establish a high-throughput phenotypic assay in hepatic stellate cells that combines fibrosis protein readouts and cytotoxicity and allows the screening of large compound libraries. To deconvolute the mode of action of the hits we also performed high-throughput transcriptomics.

Method: A high content imaging-based Pericyte-to-Myofibroblast Transition (PMT) assay was established in primary human hepatic stellate cells (HSCs), with both collagen 1 and aSMA protein readouts using different fibrosis inducers, including TGFb. The HSC-PMT assay was validated with several known anti-fibrotic compounds and was then used to screen a 50k diverse compound library in a preventive assay setup. The compound hits were confirmed and further characterized by counter assays, secondary assays, and high-throughput RNA sequencing.

Results: The screening resulted in a hit rate of ~1% and among the most potent hits we identified a compound that prevents the accumulation of collagen and aSMA in HSCs with nM to µM potency range, depending on the applied fibrosis inducer. This compound successfully passed through the counter assays (e.g. SMAD translocation assay) included in the screening cascade, and its anti-fibrotic activity was confirmed in PMT assay in pulmonary fibroblasts, indicating that this molecule targets a general fibrosis mechanism. RNA-sequencing of compound-treated cells helped elucidate the mode of action of this compound. Dimension reduction resulted in co-clustering of the hit compound with prostaglandin analogs. Furthermore, comparison of differentially expressed genes showed similarities of the hit with multi-kinase inhibitors Nintedanib and Dasatinib. Our hypothesis is that the small molecule compound inhibits several kinases, including SIKs and PDGFRs.

Conclusion: Our fully established screening cascade including the high-throughput phenotypic assay allowed the identification of a potent anti-fibrotic compound. The transcriptomics analysis facilitated a focused target deconvolution and led to the identification of several relevant kinases with anti-fibrotic activity such as SIK1/2/3 and PDGFRs.

PO2-14-YI
Loss of liver adrenoceptor alpha-1b exacerbates fibrosis and inflammation in mice

Bernie Efole[1 2], Mathilde Mouchiroud[1], Cindy Serdjebi[3], Florine Chandes[3], Andreas Lock[4], Joel K Elmquist[5], Olivier Barbier[2 6], Alexandre Caron[1 2]
[1]Quebec Heart and Lung Institute, Québec, Canada, *[2]Faculty of Pharmacy, Laval University, Québec, Canada*, *[3]Biocellvia, Marseille, France*, *[4]Althisia, Troyes, France*, *[5]UT Southwestern Medical Center, Dallas, United States*, *[6]Crchul, Québec, Canada*
Email: beefb@ulaval.ca

Background and aims: The brain influences liver metabolism through many neuroendocrine and autonomic mechanisms. Unfortunately, these sophisticated homeostatic processes can be impaired in metabolic dysfunction-associated steatotic liver disease (MASLD), in which autonomic dysfunctions including liver neuropathies have been described. However, the precise role of the autonomic nervous system in the progression of MASLD remains to be defined. Based on recent tissue clearing studies convincingly showing that neural innervations within the liver are of sympathetic nature, we hypothesized that adrenergic receptors expressed by hepatocytes play a key role in the pathogenesis of MASLD. Confirming a previous anatomical profiling study of receptor expression, we observed that the adrenoceptor alpha-1b (*Adra1b*) was the dominant subtype expressed in the liver.

Method: Using CRISPR-Cas9 technology, we developed a conditional mouse model for the *Adra1b* gene. These mice were bred with an *Albumin*-Cre mouse to generate mice lacking *Adra1b* specifically in hepatocytes (*Adra1b*-liverKO). *Adra1b*-liverKO mice and littermate controls were fed an AMLN diet containing palm oil, fructose, and cholesterol for 32 weeks to induce liver damages. Liver function tests were performed together with gene expression analysis of inflammatory markers. Whole slide images of picrosirius red- and F4/80-stained liver sections were analyzed using the fully automated, unsupervised software MorphoQuant.

Results: Despite no difference in ALT/AST between groups, *Adra1b*-liverKO had higher levels of bilirubin, and increased expression of liver inflammatory markers such as tumor necrosis factor alpha (TNF-alpha) and transforming growth factor beta (TGF-beta). Automated computerized image analysis revealed increased fibrosis and inflammation in mice lacking *Adra1b* in the liver.

Conclusion: Our data suggest that ADRA1B may have a protective role in the progression of MASLD. We believe that a better understanding of the receptors and pathways involved in the sympathetic outflow of the liver will help developing a thoughtful perspective on how the autonomic control of liver is altered in MASLD.

PO2-15
Hepatocellular loss of mTOR aggravates tumor burden in non-alcoholic steatohepatitis-related HCC

Antje Egners[1], Christian Preisinger[2], Nikolaus Berndt[3], Thorsten Cramer[1], Andreas Korh[4], Tom Florian Ulmer[4], Ulf Neumann[4], Jeanette Walter[5], Julia Andruszkow[6]
[1]University Hospital RWTH Aachen, Molecular Tumor Biology, General, Visceral and Transplantation Surgery, Aachen, Germany, *[2]University Hospital RWTH Aachen, Proteomics Facility, Interdisciplinary Center for Clinical Research (IZKF), Aachen, Germany*, *[3]German Heart Institute Berlin, Institute of Computer-assisted Cardiovascular Medicine, Berlin, Germany*, *[4]University Hospital RWTH Aachen, General, Visceral and Transplantation Surgery, Aachen, Germany*, *[5]University Hospital RWTH Aachen, Hematology, Oncology, Hemostaseology, and Stem Cell Transplantation, Aachen, Germany*, *[6]University Hospital RWTH Aachen, Pathology, Aachen, Germany*
Email: akroh@ukaachen.de

Background and aims: Obesity and associated non-alcoholic steatohepatitis (NASH) are on the rise globally. NASH became an increasingly prevalent driver of hepatocellular carcinoma (HCC) in recent years. Activation of the central metabolic regulator mTOR (mechanistic target of rapamycin) was observed in around 50% of human HCCs. However, despite promising preclinical results, mTOR inhibitors largely failed to improve the outcome of HCC therapies. This demonstrates the need for a better understanding of the molecular and functional consequences of mTOR blockade.

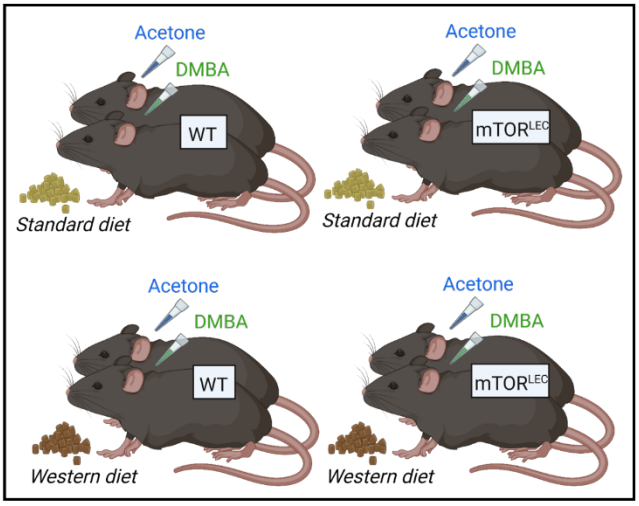
Method: We used a murine NASH-driven HCC model based on long-term western diet feeding and DMBA application combined with genetic mTOR-inactivation in hepatocytes (Alb-Cre). We evaluated tumor load and whole-body fat percentage via µCT-scans and analysed metabolic blood parameters as well as liver histology and liver tissue proteome profiles. To evaluate liver and HCC metabolic reprogramming we used a bioinformatic model of liver metabolism.

Results: mTOR-knockout massively increased the tumor burden. Interestingly, we observed improved glucose tolerance, less accumulation of visceral fat and reduced NASH-associated liver damage in mTOR-silenced mice. Analyses of liver and HCC tissue proteomics expression profiles and metabolic modelling suggest extensive reprogramming of glucose, fatty acid, bile acid and cholesterol metabolism.

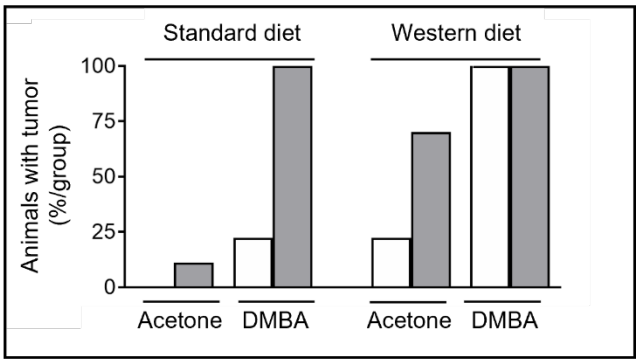
Conclusion: mTOR-inhibition in NASH-derived HCC causes extensive metabolic disturbances and elevates tumor progression. We therefore conclude that mTOR functions as tumor suppressor in hepatocytes especially under long-term western diet. However, some of the detrimental consequences of diet feeding are attenuated by loss of mTOR.

Figure:

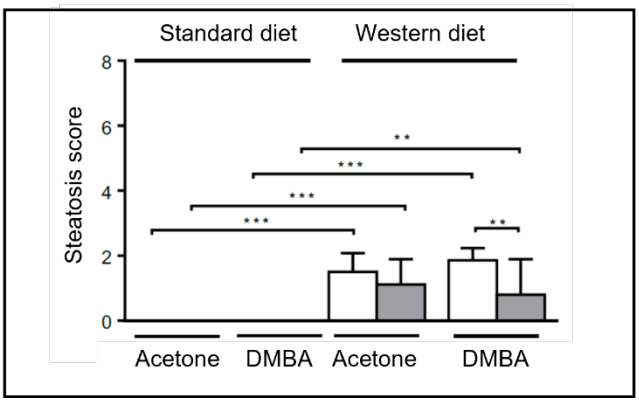
1. Experimental setup



2. Hepatocellular mTOR knockout aggravates tumor burden, especially under western diet



3. Several symptomes of metabolic syndrome are attenuated in mTOR-ko mice



PO3-03-YI
Shared genetics between NAFLD and Parkinson's disease: the role of TLR4 signaling pathway

Christina Flourou[1 2], Georgios Vavougios[3], Georgios Hadjigeorgiou[3], Chara Azina[2], Eleni Papanicolaou[2]
[1]National and Kapodistrian University of Athens, Athens, Greece, *[2]Nicosia General Hospital, Internal Medicine Department, Strovolos, Cyprus*, *[3]University of Cyprus (New Campus), Department of Neurology, Nicosia, Cyprus*
Email: christinafl@hotmail.gr

Background and aims: Given the escalating prevalence of metabolic syndrome worldwide, non-alcoholic fatty liver disease is currently considered the most common chronic liver disease in the Western world. There is a growing body of evidence that NAFLD and PD are associated on multiple levels, including molecular and potentially genetic aspects. However, to date no study has utilized high throughput omics data in order to identify genes, polymorphisms and pathways defining this relationship.

Method: We utilized the DisGeNet database to systematically retrieve categorized genes and single nucleotide polymorphisms (SNPa) characterizing NAFLD. Subsequently, we utilized the disease-disease enrichment analysis feature to identify gene and SNP overlaps between NAFLD and Parkinson's disease. Common genes were examined via pathway enrichment analysis, and shared SNPs were investigated using SNPs enrichment analysis. For these enrichment analyses, a false discovery rate (FDR) of less than 0.05 was deemed statistically significant.

Results: A total of one thousand fifty-eight (1058) genes and two hundred twenty-two (222) single nucleotide polymorphisms (SNPs) were associated with NAFLD, with a protein-protein interaction (PPI) enrichment p value of less than 1.0e-16. The genetic comparison between NAFLD and Parkinson's disease revealed that four hundred seven (407) genes and fifteen SNPs were common to both. One significantly enriched pathway was the TLR4 signaling pathway. Genetic defects in the TLR4 signaling pathway in both diseases were correlated with a specific single nucleotide polymorphism (SNP), rs4986791. [1]

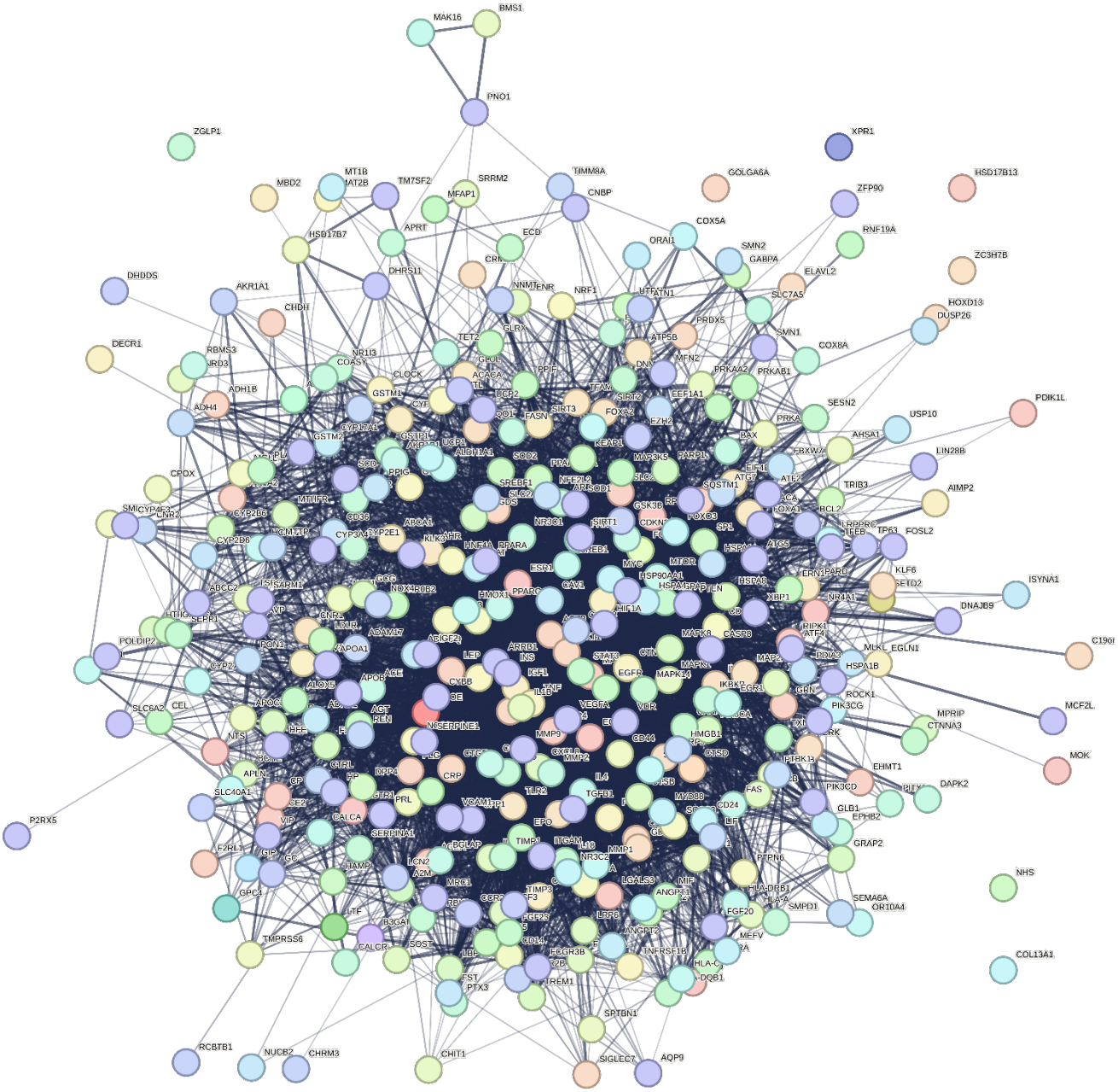
Conclusion: Multiple immune and metabolic pathways are linked with both NAFLD and Parkinson's disease. This genome and polymorphisms analysis has highlighted the overlap between NAFLD and PD. Importantly, while genetic and epigenetic defects in TLR4 have been independently associated with either NAFLD or PD, ours is the first report to associate TLR4 with both diseases, as well as a specific TLR4 polymorphism, SNP rs4986791.

Polymorphisms that activate Toll-like receptor 4 subsequently trigger the TLR4/TNF-a signaling pathway. Specifically, by mediating the TLR4/TBK1/NF-jB/TNF-a signaling pathway, inflammation induces peripheral immune activation. Inflammatory factors cross the blood brain barrier, causing neuroinflammation and reduced levels of dopamine (DA) and 5-hydroxytryptamine (5-HT). [2]
In NAFLD, lipotoxicity prompts hepatocyte death, leading to the release of death-associated molecular patterns (DAMPs). This initiates the TLR4 pathway activating the nuclear factor (NF)-kB and releasing chemokines. Subsequent secretion of TGF-β and tissue inhibitor of metalloproteinase results in liver fibrosis and disease progression. [3]

In both NAFLD and Parkinson's disease, the microbiota-gut-brain axis plays a significant role. The expression of LPS molecules on Gram negative bacteria and the bacterial translocation, all activate TLR4. In the first instance, LPS molecules on Gram negative bacteria are recognized by CD14 and transferred to the MD2-TLR4 complex, which activates the TLR4 pathway. [4]

Despite of the multiple genetic, epigenetic and environmental factors that impact on both diseases' expression, the TLR4 pathway through this specific polymorphism, the SNP rs4986791, seems to be a sign for the coexistence of diseases, early diagnosis, intervention and also a therapeutic goal.

Figure: NAFLD pathways [5]



PO3-13-YI
CTRP1, a novel molecular linker for hepatic glycogen and lipid metabolism ameliorates fatty liver diseases

Sora Han[1], Young Yang[1]
[1]Sookmyung Women's University, Seoul, Research Institute of Women's Health, Seoul, Korea, Rep. of South
Email: ddoie@sookmyung.ac.kr

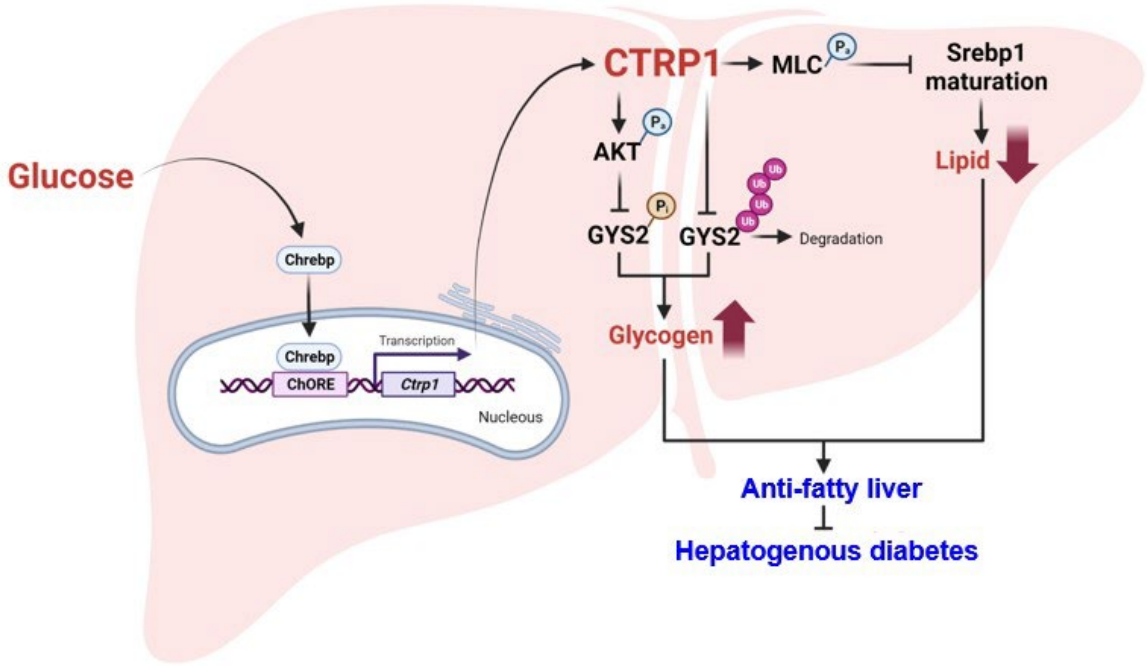
Background and aims: An adiponectin paralog, C1q/TNF-alpha-related protein 1 (CTRP1) is known to enhance glucose and fatty acid utilization by modulating the activities of IRS-1, AKT, AMPK, ACC, and HSL in skeletal muscles and adipocytes, which ameliorates systemic hyperglycemia and insulin resistance in mouse and cell line models. Circulating CTRP1 levels were found to be significantly elevated in obese hyperglycemic adolescents and patients with obesity and type 2 diabetes mellitus (T2DM), suggesting an association CTRP1 with these metabolic diseases. Interestingly, we previously observed histologically that global overexpression CTPR1 in mice resulted in a significant increase in hepatic glycogen content. However, the mechanism through which CTRP1 controls hepatic glycogen remains unknown, prompting us to investigate the metabolic functions of CTRP1 in the livers in association with T2DM.

Method: Regulatory functions of CTRP1 in the livers were elucidated mainly using tamoxifen-inducible CTRP1 conditional knockout mouse (KO), primary hepatocytes, cell lines, and CTRP1-overexpressing adenovirus and plasmids. Circulating CTRP1 levels were analyzed to assess the clinical association of CTRP1 and fatty liver diseases.

Results: We have demonstrated that CTRP1 is a novel downstream target of ChREBP, controls both glycogen synthesis and lipid accumulation in the liver, thereby ameliorating fatty liver and systemic insulin resistance. Mechanistically, CTRP1 enhances hepatic glycogen levels by increasing both insulin-dependent glycogenic activity and the protein stability of glycogen synthase 2. Additionally, CTRP1 was found to decrease the protein maturation of the lipogenic transcription factor, Srebp1, which suppresses *de novo* lipogenesis. CTRP1 KO with hepatic glycogen depletion and fatty liver exhibit insulin resistance and impaired glucose tolerance on a high-carbohydrate drink, mimicking the clinical symptoms of patients with pre-T2DM. Notably, circulating CTRP1 levels were significantly correlated with the progression of hepatitis to cirrhosis in patients with T2DM, indicating the possibility that the dual role of CTPR1 in regulating hepatic glycogen and lipid attributes to both physiological and pathophysiological process of hepatogenous diabetes.

Conclusion: CTRP1 would be a potential molecular link between hepatic glycogen and lipid balance and the development of systemic insulin resistance and T2DM.

Figure:


Glucose
Chrebp
Chrebp
ChORE
Transcription
Ctrp1
Nucleous
CTRP1
MLC
Srebp1
maturation
AKT
Lipid
GYS2
GYS2
Degradation
Glycogen
Anti-fatty liver
Hepatogenous diabetes

PO3-15
Mechanisms underlying steatotic liver disease in mental illness: antipsychotic drugs disrupt ferroptosis signaling pathways

Karen Houseknecht[1], Deborah Barlow[1], Calvin Vary[2], Meghan May[1]
[1]University of New England, Biomedical Sciences, Biddeford, United States, *[2]MaineHealth Institute for Research, Scarborough, United States*
Email: khouseknecht@une.edu

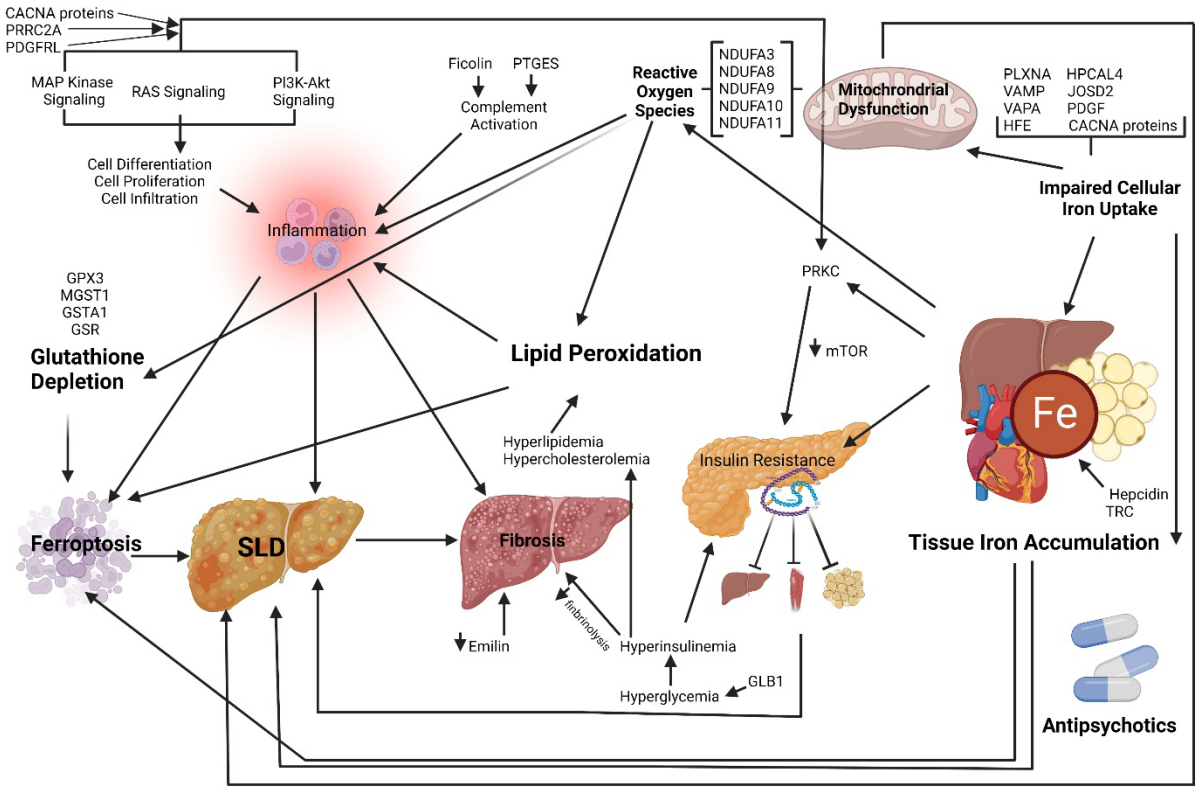
Background and aims: Steatotic liver disease (SLD) occurs in obese, non-obese and lean people, and is increased with mental illness. Myriad factors contribute to increased SLD risk in this patient population, including psychiatric drugs. Mechanisms underlying antipsychotic (AA) induced metabolic liver disease are poorly understood. Ferroptosis, programmed cell death linked to disrupted iron (Fe) metabolism, lipid peroxidation, and glutathione (GSH) depletion, has been implicated in SLD disease incidence and progression. The impact of AA therapy on ferroptosis is not understood. We hypothesize that AA alter hepatic signaling pathways that regulate ferroptosis, leading to increased SLD in drug treated individuals. Our aim was to examine the role of ferroptosis in the development of AA induced SLD using proteomic and bioanalytical methodologies in a pre-clinical model.

Method: C57BL/6J mice (8 wk) were fed chow diet and treated with risperidone (RIS, 1 mg/kg PO), olanzapine (OLAN, 5 mg/kg PO) or drug vehicle (VEH, 0.1% acetic acid PO) for 4 weeks. Drug doses were chosen to achieve peak plasma drug concentrations within the clinical range. Hepatic proteomes were analyzed by mass spectrometry and profiled by sequential window acquisition of all theoretical spectra (SWATH) analysis. Differentially expressed (DE) proteins were identified (significance $p < 0.05$) and mapped to relevant disease pathways (KEGG). SLD was histologically confirmed. Hepatic Fe concentrations and plasma ferritin were measured using commercially available reagents (Sigma Aldrich, Abcam). Prostaglandin species (PGE and PGF) were quantified by LC/MSMS.

Results: There was no effect of AA on bodyweight, however AA treatment caused histopathology confirmed SLD in the absence of obesity in mice. Liver Fe was increased with AA ($p < 0.01$) and Fe^{3+} was increased by RIS ($p < 0.05$) but not OLAN. Plasma ferritin was not altered by AA ($p > 0.05$). Proteomic analysis revealed AA-associated changes in known SLD pathways. Ferroptosis associated traits were differentially expressed (DE) with AA ($p < 0.05$). Reactive oxygen species (ROS) signaling was a target of AA action (34 proteins up-regulated, 10 down-regulated for RIS; 17 up-regulated and 3 down regulated for OLAN) as were proteins involved in mitochondrial function (5 NDUF proteins). Proteins in glutathione metabolic pathways including GPX3, MGST1, G6PD and GSTA1 were DE with RIS ($p < 0.04$). Mitochondrial GSR was reduced by OLAN ($p < 0.03$). DE traits associated with Fe metabolism and anemia were identified in liver proteomes from RIS mice including HFE and TFRC ($p < 0.04$).

Conclusion: SLD incidence is increased in mental illness and with antipsychotic treatment, clinically and pre-clinically. AA associated SLD occurs in the presence and absence of obesity and is linked to disrupted hepatic Fe metabolism and DE traits pivotal to ferroptosis signaling. These data support the hypothesis that AA drugs act, at least in part, by altering ferroptosis pathways in SLD.

Figure:



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PO4-06-YI
Unleashing the potential of phytocannabinoids in modulating sphingolipid homeostasis for the treatment of metabolic dysfunction-associated steatotic liver disease

Radka Kocvarova[1], Shahar Azar[1], Yossi Tam[1], Ifat Abramovich[2], Eyal Gottlieb[2], Bella Agranovich[2]
[1]The Hebrew University of Jerusalem, School of Pharmacy, Faculty of Medicine, Jerusalem, Israel,
[2]The Ruth and Bruce Rappaport Faculty of Medicine, Rappaport Faculty of Medicine and Research Institute, Haifa, Israel
Email: radka.kocvarova@mail.huji.ac.il

Background and aims: Metabolic dysfunction-associated steatotic liver disease (MASLD), a prevalent condition associated with obesity, poses a substantial burden on global health. With the liver playing a central role in metabolism, its vulnerability to the detrimental effects of obesity calls for innovative therapeutic approaches. Phytocannabinoids, naturally occurring compounds found in the cannabis plant, have garnered attention for their potential in addressing metabolic abnormalities, including weight reduction, improved insulin sensitivity, and modulation of hepatic lipid metabolism. However, a comprehensive understanding of the therapeutic potential of specific non-psychotropic phytocannabinoids, such as cannabidiol (CBD) and cannabigerol (CBG), in combating MASLD is still unfolding.

Method: This study aimed to investigate the therapeutic potential of CBD (5 mg/kg/day, IP) and/or CBG (12.5 mg/kg/day, IP) in a high-fat diet (HFD)-induced obese mouse model. Through a multifaceted approach encompassing biochemical, histological, and in vivo assessments, we explored the impact of CBD and/or CBG on hepatic steatosis, dyslipidemia, and body weight gain. Furthermore, leveraging advanced high-throughput metabolomics analysis using LC-MS technology, we aimed to elucidate the underlying molecular mechanisms by which both phytocannabinoids affected liver homeostasis and metabolism.

Results: The study uncovered compelling results highlighting the therapeutic potential of phytocannabinoids in the management of MASLD. CBD, CBG, and the CBD+CBG combination demonstrated significant attenuation of HFD-induced hepatic steatosis. Intriguingly, all treatment groups exhibited improvements in hypercholesterolemia and hypertriglyceridemia, irrespective of changes in body weight. Notably, CBG treatment resulted in a pronounced reduction in fat mass and an increase in lean mass. Metabolomics analysis of liver tissue unveiled substantial alterations in metabolites associated with diverse metabolic pathways, prominently implicating sphingolipid modulation as a key anti-steatotic mechanism influenced by phytocannabinoids.

Conclusion: Our study demonstrates the tangible therapeutic potential of phytocannabinoids, particularly CBD and/or CBG, in effectively managing MASLD. The significant reduction in HFD-induced hepatic steatosis, improvements in hypercholesterolemia and hypertriglyceridemia, and the modulation of sphingolipid metabolism observed in our research provide concrete evidence of their beneficial effects on liver health. By targeting sphingolipid modulation, phytocannabinoids offer a promising avenue for developing precise and targeted interventions against obesity-related MASLD. Further investigation and clinical validation of these findings are essential for translating them into effective treatments.

Figure:

PO4-11-YI
In silico strategies and gene expression data indicate EFEMP1 as a potential biomarker for early fibrosis diagnosis in metabolic dysfunction-associated steatotic liver disease

Allen Anthony Laraño[1 2 3], Deborah Bonazza[4 5], Silvia Palmisano[5 6], Claudio Tiribelli[1], Natalia Rosso[1], Pablo J Giraudi[1]
[1]Metabolic Liver Disease Unit, Fondazione Italiana Fegato (Italian Liver Foundation), Trieste, Italy, *[2]Department of Life Sciences, University of Trieste, Trieste, Italy*, *[3]Philippine Council for Health Research and Development, DOST Compound, Taguig City, Philippines*, *[4]Surgical Pathology Unit, Cattinara Hospital, ASUGI, Trieste, Italy*, *[5]Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy*, *[6]Surgical Clinic Division, Cattinara Hospital, ASUITs, Trieste, Italy*
Email: allenanthony.larano@fegato.it

Background and aims: A quarter of adults worldwide suffer from metabolic dysfunction-associated steatotic liver disease (MASLD). Fibrosis is among the best predictors of liver-related risk in persons with MASLD. Although liver biopsy is still the gold standard for MASLD diagnosis, non-invasive diagnostic tools are crucial in detecting fibrosis, especially in severely obese subjects where non-invasive procedures are not readily available. We aim to identify, using *in silico* methods, circulating biomarkers associated with early fibrosis development and validate their specificity in a MASLD cohort.

Method: RNAseq data GSE125251 was retrieved from the NCBI GEO repository and re-analyzed using the 3DRNAseq analysis pipeline; differentially expressed genes (DEGs) were determined from liver transcriptomes comparison of F0-F1 (early fibrosis, n = 139) vs. F2-F3-F4 (moderate/advanced fibrosis, n = 67) MASLD subjects. Gene-set enrichment analysis was performed to determine DEGs' involvement in molecular pathways. DEGs were filtered, selecting secreted plasma protein-coding genes through an *in-silico* funnel strategy. Gene expression of candidates was analyzed in liver samples of a MASLD biopsy-proven cohort (n = 65) stratified according to minimal (FO/F1) and moderate/advanced fibrosis (F2/F3-F4).

Results: The re-analysis of GSE125251 identified 106 DEGs when comparing moderate vs. early fibrosis. Pathway enrichment analysis demonstrated that most DEGs were associated with pathways involved in fibrogenesis such as extracellular matrix production (ECM) and the inflammatory response. Twenty-two DEGs encoded secreted proteins and were categorized as proteins detectable in plasma using the *in-silico* strategy. Five candidates (*EFEMP1, LTBP2, LUM, DPT, CCL20*) were analyzed at the mRNA level. Among them, *EFEMP1* (EGF-containing fibulin extracellular matrix protein 1) showed the highest change, exhibiting increased mRNA expression levels in F2/F3-F4 fibrotic livers when compared with (F0/F1 -ones (p value <0.005).

Conclusion: Based on this pilot study, EFEMP1 seems to be the best candidate for future validation as a circulating fibrosis biomarker in MASLD. EFEMP1 is noted to be a secreted ECM protein, normally expressed by fibroblasts, and showing an important role in maintaining ECM stability and integrity. Further protein-level analyses are necessary to confirm the utility of EFEMP1 as a biomarker of liver fibrosis in MASLD.

Figure:

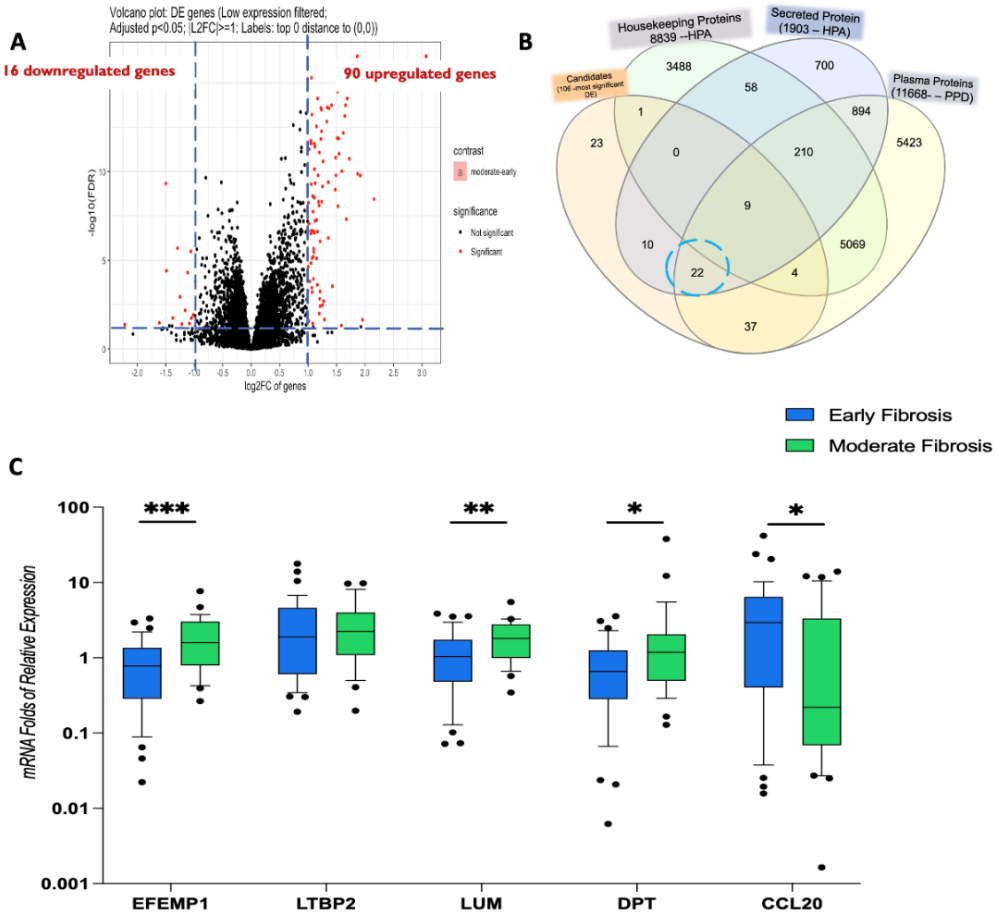


Figure 1: A) Volcano plot illustrating the differentially expressed genes in fibrotic samples of the GSE125251 dataset. B) Venn diagrams showing the number of DEGs identified as candidates by the *in-silico* funnel strategy. C) mRNA liver expression levels of 5 random selected candidates. Data are shown as mean ± SD. Group comparison by Kruskal-Wallis and post hoc Dunn's test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

PO4-14-YI
Apolipoprotein F deficiency is associated with reduced mitochondrial function in hepatocytes

Viktor Lienard[1], Audrey Deprince[1], Céline Gheeraert[1], Nathalie Hennuyer[1], Pauline Jacquemain[1], Niels Kloosterhuis[2], Eeckhoute Jerôme[1], Bart van de Sluis[2], Bart Staels[1], Delphine Eberlé[1], Joel Haas[1]
[1]Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1011, EGID, Lille, France, *[2]Dept. Paediatrics, Univ. Groningen, University Medical Center Groningen, Netherlands*
Email: viktor.lienard@univ-lille.fr

Background and aims: Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD, previously NAFLD) is the most common chronic liver disease worldwide, with its prevalence rising in concert with rates of obesity. MASLD is characterized primarily by hepatic triglyceride (TG) accumulation, called steatosis, which can progress to metabolic dysfunction-associated steatohepatitis (MASH), which combines steatosis with elements of hepatic necroinflammation. Our group recently reported that hepatic *APOF* (encoding Apolipoprotein F) mRNA expression inversely correlates with steatosis in humans, and its expression is reduced by half in patients with MASH. We also found that ApoF overexpression in mice enhanced hepatic clearance of remnant lipoproteins. Therefore, our aim is to determine if reduced ApoF expression contributes to progression from steatosis to MASH or associated complications.

Method: We established a new mouse model deficient for *ApoF* (ApoF-KO). ApoF-KO mice and littermate controls were fed a high-fat diet supplemented with sucrose and cholesterol (HFSC diet) for 22 weeks. After sacrifice, hepatic lipids were measured and gene expression was analyzed by RNA sequencing. We measured mitochondrial function in primary hepatocytes from chow-fed ApoF-KO mice and littermate controls with the Seahorse Extracellular Flux Analyzer.

Results: No changes in body weight, plasma lipids and glycemia were observed between ApoF-KO and control mice on HFSC diet. However, hepatic TG and cholesterol contents were lower in ApoF-KO compared to controls (-57%, p = 0.047, and -25%, p = 0.129 respectively), suggesting that *ApoF* deficiency protects from hepatic lipid accumulation. Liver transcriptomic analysis revealed decreased expression of genes related to mitochondrial function (i.e. fatty acid catabolism, oxidative phosphorylation) and structure (*Ndufa9, Ndufaf1, Ndufaf5, Sdha, Sirt4* etc*)* in ApoF-KO mice compared to control mice on HFSC diet. Finally, primary hepatocytes from chow-fed ApoF-KO mice displayed decreased mitochondrial function (notably decreased spare respiratory capacity) compared to littermate controls, further supporting a role for *ApoF* in the control of hepatic mitochondrial metabolism.

Conclusion: Our results indicate that *ApoF* deficiency impacts hepatic mitochondrial function and lipid accumulation in the context of MASLD. Further studies are necessary to elucidate the link between *ApoF* expression and mitochondrial function.

PO5-05
Transcriptomics driven metabolic pathway analysis to explore metabolic resemblance between metabolic-dysfunction associated steatohepatitis mouse models and human

Sofia Tsouka[1], Pavitra Kumar[2], Patcharamon Seubnooch[1], Jean-François Dufour[2], Mojgan Masoodi[1] [1]
[1]University Hospital Bern, Institute of Clinical Chemistry, Bern, Switzerland, *[2]University Bern, Bern, Switzerland*
Email: mojgan.masoodi@insel.ch

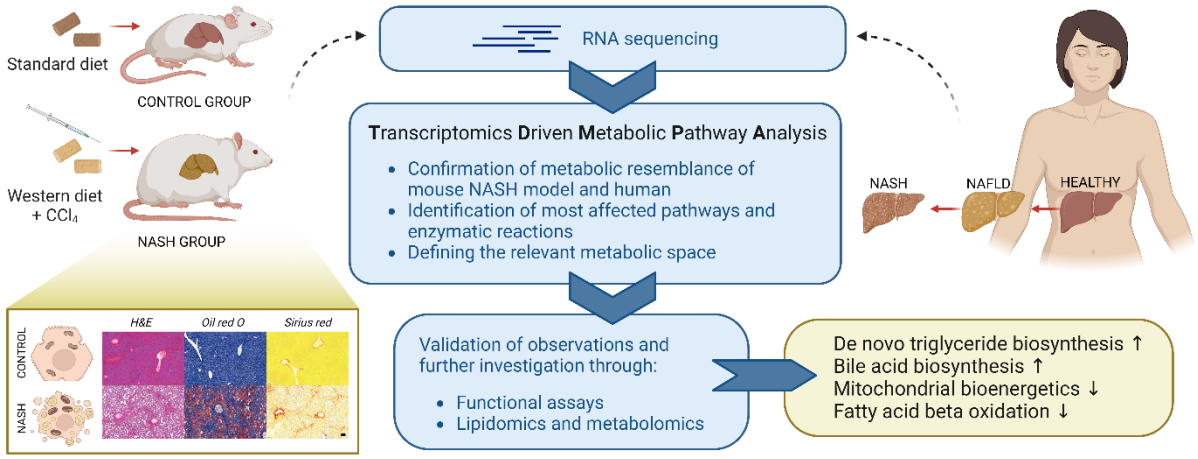
Background and aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a prevalent chronic liver disease worldwide, and can rapidly progress to metabolic dysfunction-associated steatohepatitis (MASH). Accurate preclinical models and methodologies are needed to understand underlying metabolic mechanisms and develop treatment strategies.

Method: We developed transcriptomics driven metabolic pathway analysis (TDMPA), a method that uses genome-scale metabolic models to calculate enzymatic reaction perturbations from gene expression data. We used TDMPA to score and compare metabolic pathway alterations in MASH mouse models to human MASH. We developed an already established WD+CCl4-induced MASH model and performed functional assays and lipidomics to confirm TDMPA findings.

Results: Human MASH and the mouse model both exhibit numerous altered metabolic pathways, including triglyceride biosynthesis, fatty acid beta-oxidation, bile acid biosynthesis, cholesterol metabolism, and oxidative phosphorylation. We confirmed significant reduction in mitochondrial functions and bioenergetics in MASH model, and in acylcarnitines. We identified a wide range of lipid species within the most perturbed pathways predicted by TDMPA. Triglycerides, phospholipids and bile acids were increased significantly in MASH, confirming our initial observations.

Conclusion: We introduced TDMPA, a methodology for evaluating metabolic pathway alterations in metabolic disorders. By comparing metabolic signatures that typify human MASH, we evaluated metabolic resemblance of the mouse model to human. Our presented approach provides a valuable tool for defining metabolic space to aid experimental design for assessing metabolism.

Figure:



PO5-07-YI
Hypoxanthine plasma levels as a new potential non-invasive biomarker of metabolic-dysfunction associated steatotic liver disease

Melissa Maria Milito[1], Pablo J Giraudi[1], Natalia Rosso[1], Claudio Tiribelli[1], Deborah Bonazza[2], Silvia Palmisano[3]
[1]Fondazione Italiana Fegato Onlus, Basovizza, Italy, *[2]Cattinara Hospital, Surgical pathology unit, Trieste, Italy*, *[3]University of Trieste, Department of Medical, Surgical and Health Sciences, Trieste, Italy*
Email: melissa.milito@fegato.it

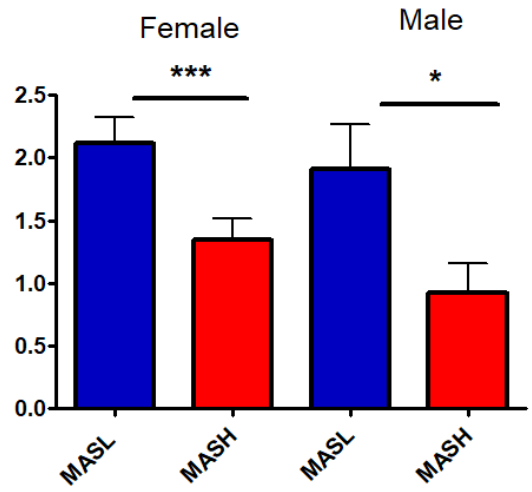
Background and aims: Metabolic-dysfunction steatotic liver disease (MASLD) is strongly associated with obesity and a cluster of metabolic disorders. Different diagnostic modalities are used to detect MASLD such as ultrasonography, but it has low sensitivity in severely obese subjects. Thus, liver biopsy remains the gold standard. New minimally invasive methods such as plasma-based biomarker tests are therefore needed. This study aims to establish an initial validation of the putative use of plasma hypoxanthine (Hypx) as a marker of metabolic-dysfunction associated steatohepatitis (MASH). Specifically, to characterize hypoxanthine pool changes in blood, liver, and Visceral Adipose Tissue (VAT) and to evaluate the main factors (enzyme and genes) involved in purine catabolism.

Method: Plasma Hypx was quantified using a fluorometric enzymatic reaction-based kit in 132 biopsy-proven bariatric subjects stratified according to fatty liver (MASL) and MASH. Hypx concentration was also determined in liver and VAT homogenates. Correlation analyses were performed among plasma Hypx level and several clinical-biochemical-histological parameters. Additionally, correlation analysis was performed among plasma Hypx and its intracellular concentrations in liver and VAT tissue homogenates. Gene expression analysis of *XDH, HPRT, and PNP* which are involved in purine metabolism was performed in a subgroup of liver and VAT samples (n = 56). Xanthine Oxidoreductase enzyme (XDH/XOR) activity, the rate-limiting enzyme of the purine catabolism cycle, was also measured.

Results: Hypoxanthine concentration was reduced in plasma of MASH patients (MASL Hypx 2.0 [IQR 1.16-3.0] vs. MASH Hypx plasma Median: 0.8 [IQR 0.5-1.9] *p value <0.0001*), independent of sex. An AUC value of 0.75 was observed by performing receiver operating characteristics (ROC) curve analysis. On the other hand, no difference in intracellular Hypx concentrations was observed in liver and VAT homogenates. Interestingly, correlation analyses indicated that Hypx levels correlate with lobular inflammation (rho = -0.23; *p = 0.010*), ballooning grade (rho = -0.48*; p <0.0001*), fibrosis stage (rho = -0.33; *p = 0.0006*), GGT (rho = 0.18; *p = 0.04*), and triglycerides (rho = 0.22; *p = 0.017*). Gene expression level showed significant changes in the VAT of MASH patients (*XDH*: 18.0 ± 13.2 MASL vs 1.5 ± 9.0 MASH; *p = 0.003* ; *PNP*: 0.3 ± 0.6 MASL vs. 1.3 ± 0.9 MASH, *p = 0.003*; *HPRT*: 11.4 ± 13.7 MASL vs. 1.8 ± 6.2 MASH, *p = 0.007*), while no differences were observed in XOR enzyme activity between MASL and MASH in both plasma and tissue levels.

Conclusion: Our findings suggest that hypoxanthine plasma levels in MASLD patients could be used as a minimally invasive biomarker for the identification of patients with MASH. Moreover, our results indicate that VAT (or another organ/tissue) might contribute to the hypoxanthine changes observed in MASH patients. Further studies are required to determine its utility as a diagnostic biomarker.

Figure: Hypoxanthine concentration detected in plasma samples of MASL and MASH patients. Significant at * *p <0.05*, *** *p <0.0001*



PO6-02-YI
Fructose induced perturbation in hepatic proteostasis via ribosomal protein S6 kinase beta-1 (S6K1) activation contributes to de novo lipogenesis in non-alcohol-related fatty liver disease

Sana Raza[1], Rohit Sinha[2]
[1]Sanjay gandhi posgtraduate institute of medical sciences, Endocrinology, Lucknow, India, *[1]Sanjay gandhi posgtraduate institute of medical sciences, Endocrinology, Lucknow, India*
Email: raza.sana9@gmail.com

Background and aims: The dysregulation of hepatic lipid metabolism remains a common denominator in the development of non-alcohol-related fatty liver disease (NAFLD). With the dramatic increase in dietary fructose intake through increased consumption of refined sugar worldwide, fructose has emerged as an inducer of hepatic de novo lipogenesis (DNL), a major contributor to NAFLD. This study aimed to investigate the molecular mechanism underlying fructose induced endoplasmic reticulum (ER) stress and fatty acid synthesis in NALFD to discover new anti-NAFLD drug targets.

Method: Transcriptomics analysis was performed using a public human NAFLD microarray dataset E-MEXP-3291 comprising of 45 patients (Normal, steatosis and non-alcoholic steatohepatitis (NASH)). To investigate the effects of fructose on lipid accumulation, HepG2 cells were stimulated with fructose (20 mmol/L) for 6, 12, or 24hr. DNL was assessed by analyzing the mRNA expression of fatty acyl synthase (FAS). Lipid accumulation was assessed by Oil Red O staining. Fructose induced protein synthesis, ER stress, and autophagic flux were analyzed by Protein Synthesis Assay and Western Blot analysis of protein synthesis, ER stress and autophagy markers. Cycloheximide (CHX), a protein synthesis inhibitor, and PF4708671, a cell-permeable inhibitor of p70 ribosomal S6 kinase were used to study the effect of fructose induced protein synthesis on ER stress and DNL.

Results: Human microarray data indicated the upregulation of hepatic protein synthesis pathways in NAFLD patients. Using HepG2 cell model of hepatic steatosis, fructose stimulation (6hr) increased the expression of p70S6K, S6K1, pEIF4B inducing protein synthesis and enhanced the mRNA expression of FAS, an upstream regulator of DNL. Fructose induced DNL was reduced upon inhibition of protein synthesis. Fructose induced protein synthesis activated unfolded protein response (UPR) and ER stress by inducing X-box binding protein1 (XBP1) and phosphorylated eukaryotic translation initiation factor (EIF)2-alpha, and polyubiquitinated protein expression. Fructose induced increase in protein synthesis and DNL was reduced by cell specific inhibition of S6K1. Fructose induced activation of mTOR signaling and autophagy inhibition contributes to ER stress via positive regulation of S6K1.

Conclusion: Our results underpin the critical role of fructose induced proteostasis defect as a major contributor to hepatic lipogenesis and steatosis. At a molecular level, S6K1 driven translation overdrive coupled with autophagy defects contributes to ER stress and resulting lipogenic gene transcription, in response to fructose administration in hepatic cells. Our study, thus, proposes the use of selective S6K1 inhibitors to counter ER stress and hepatic steatosis associated with NAFLD.

PO6-04-YI
miR-21-5p promotes MASH-related hepatocarcinogenesis

Pedro Miguel Rodrigues[1 2 3 4], Marta B. Afonso[1], André L. Simão[1], Tawhidul Islam[1], Maria Manuela Gaspar[1], Colm O Rourke[5], Monika Lewinska[5], Jesper Andersen[5], Enara Arretxe[6], Cristina Alonso[6], Alvaro Santos-Laso[2], Laura Izquierdo-Sánchez[2 3], Raul Jimenez-Aguero[2], Emma Eizaguirre[2], Luis Bujanda[2 3], María Jesús Pareja[7], Carina Prip-Buus[8], Jesus Maria Banales[2 3 4 9], Cecília M. P Rodrigues[1], Rui E. Castro[1]
[1]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal, *[2]Biodonostia Health Research Institute, Liver Diseases Group, San Sebastian, Spain*, *[3]Centre for the Study of Liver and Gastrointestinal Diseases (CIBERehd), Carlos III National Institute of Health, Madrid, Spain*, *[4]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain*, *[5]Biotech Research and Innovation Centre, Department of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark*, *[6]OWL Metabolomics, Bilbao, Spain*, *[7]Hospital Juan Ramón Jiménez, Huelva, Spain*, *[8]Université Paris Descartes UMR-S1016, Institut Cochin, Paris, France*, *[9]University of Navarra, Department of Biochemistry and Genetics, School of Sciences, Pamplona, Spain*
Email: pedro.rodrigues@biodonostia.org

Background and aims: The mechanisms governing the progression of metabolic dysfunction-associated steatotic liver disease (MASLD) towards steatohepatitis (MASH) and hepatocellular carcinoma (HCC) remain elusive. Here, we evaluated the role of hsa-miRNA-21-5p in MASH-related hepatocarcinogenesis.

Method: Hepatic hsa-miR-21-5p expression was evaluated in 2 cohorts of patients with biopsy-proven MASLD (n = 199) or HCC (n = 366 HCC and n = 11 MASLD-HCC). Serum/liver metabolomic profiles were correlated with hsa-miR-21-5p in MASLD obese patients. Wild-type (WT) and Mir21 KO mice were fed a choline-deficient, amino acid-defined (CDAA) diet for 32 and 66 weeks to induce MASH and MASH-HCC, respectively.

Results: In obese individuals, hsa-miR-21-5p expression increased with MASLD severity and associated with a hepatic lipotoxic profile. CDAA-fed WT mice displayed increased hepatic mmu-miR-21-5p levels and progressively developed MASH and fibrosis, with livers presenting macroscopically-discernible pre-neoplastic nodules, hyperplastic foci and deregulated cancer-related pathways. Mir21 KO mice exhibited peroxisome-proliferator activated receptor α (PPARα) activation, augmented mitochondrial activity, reduced liver injury and NAS below the threshold for MASH diagnosis, with the pro-inflammatory/fibrogenic milieu reversing to baseline levels. In parallel, Mir21 KO mice displayed reduced number of pre-neoplastic nodules, hepatocyte proliferation and activation of oncogenic signaling, being protected from MASH-associated carcinogenesis. The hsa-miRNA-21-5p/PPARα pathway was similarly deregulated in patients with HCC or MASH-related HCC, correlating with HCC markers and worse prognosis.

Conclusion: hsa-miR-21-5p is a key inducer of whole-spectrum MASLD progression, from simple steatosis to MASH and MASH-associated carcinogenesis. Inhibition of hsa-miR-21-5p, leading to a pro-metabolomic profile, might constitute an appealing therapeutic approach to ameliorate MASH and prevent progression towards HCC.

PO6-08-YI
The potential role of Omentin-1 in metabolic-dysfunction associated steatotic liver disease (MASLD): evidence from translational studies

Noel Salvoza[1 2 3], Pablo J Giraudi[1], Silvia Gazzin[1], Deborah Bonazza[4], Silvia Palmisano[1 5], Nicolò de Manzini[5], Fabrizio Zanconati[4], Alan Raseni[6], Francesca Sirianni[6], Claudio Tiribelli[1], Natalia Rosso[1]
[1]Fondazione Italiana Fegato Onlus, Basovizza, Italy, *[2]Università di Trieste-Dipartimento di Scienze della Vita, Trieste, Italy*, *[3]Philippine Council for Health Research and Development, Taguig, Philippines*, *[4]Cattinara Hospital, Surgical Pathology Unit, Trieste, Italy*, *[5]University of Trieste, Department of Medical, Surgical and Health Sciences, Trieste, Italy*, *[6]Children's hospital "Burlo Garofolo", Clinical Chemistry Urgency Laboratory Spoke, Trieste, Italy*
Email: noel.salvoza@fegato.it

Background and aims: Obesity, characterized by excessive visceral adipose tissue (VAT) is tightly associated with metabolic-dysfunction associated steatotic liver disease (MASLD). The pathogenesis of MASLD is complex but recent studies reveal that the adipose tissue-liver axis plays a key role in MASLD development. We investigated the potential role of omentin-1, a novel adipokine expressed by VAT, in MASLD pathogenesis.

Method: Through *in silico* analysis of differentially expressed genes in VAT from obese patients with and without metabolic-dysfunction associated steatohepatitis (MASH), we identified omentin-1 as a significant candidate. To validate our findings, we measured omentin-1 levels in VAT and plasma of lean controls and obese patients with biopsy-proven MASLD. Additionally, we assessed omentin-1 expression in the VAT of a juvenile mice MASLD model. *In vitro* and *ex vivo* studies were conducted to investigate the effects of omentin-1 on MASLD-related mechanisms, including steatosis, inflammation, ER stress, and oxidative stress. Finally, the effects of D-glucose and insulin on VAT omentin-1 were also analyzed *ex vivo*.

Results: The obese groups showed significantly lower VAT mRNA expression and plasma levels of omentin-1 as compared to the lean group (all p values <0.05). Interestingly, within the MASH group, fibrosis does not affect omentin-1 expression. Likewise, VAT of mice fed with high-fat diet, showing histological signs of MASH showed decreased omentin-1 mRNA and protein expression as compared to their control diet counterpart (all p values <0.05). *In vitro*, the addition of omentin-1 on fat-loaded (FFA) human hepatocytes showed no effect on steatosis but significantly decreased TNF-alpha levels (mRNA and protein), reduction in ER stress markers (*BiP* and *Chop*), and enhanced superoxide dismutase (SOD) antioxidant activity (all p values <0.05 *vs.* FFA). The same results were obtained using *ex vivo* VAT explants from obese patients upon omentin-1 supplementation (all p values <0.05 *vs.* control). In addition, omentin-1 reduced nuclear factor kappa B (*NF-KB*) mRNA expression in both *in vitro* (p value <0.01 *vs.* FFA) and *ex vivo* (p value <0.01 *vs.* control) studies. In VAT explants, D-glucose and insulin significantly reduced omentin-1 mRNA expression and protein levels (all p values <0.05 *vs.* control).

Conclusion: Collectively, our findings suggest that reduced omentin-1 levels contribute to the development of MASLD. Omentin-1 supplementation mitigates inflammation, ER stress, and oxidative stress, probably via inhibiting the NF-κB pathway and might also play a role in the regulation of glucose and insulin metabolism. Further research is warranted to explore omentin-1 as a potential therapeutic target and/or biomarker for MASLD.

Figure:

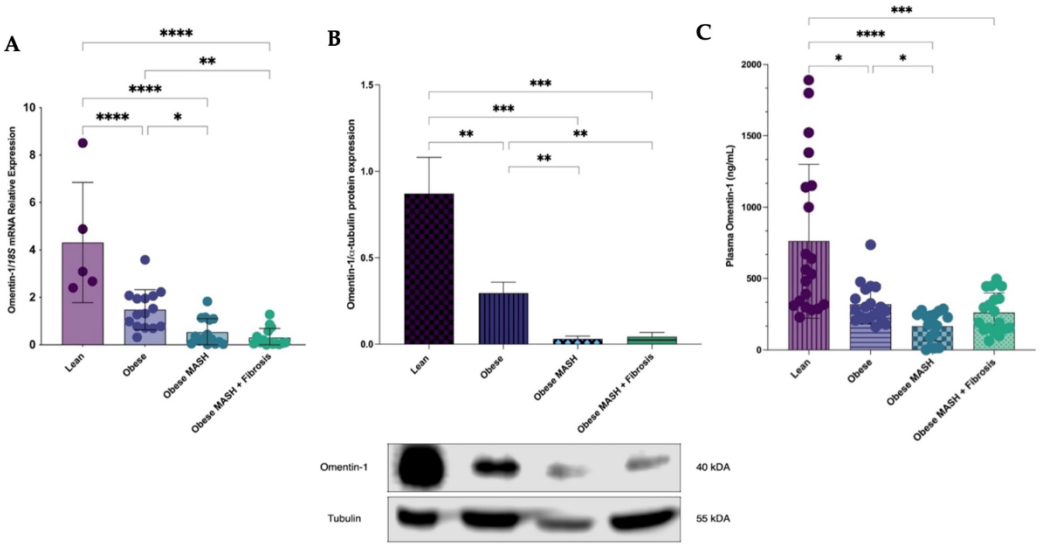


Figure 1. Human VAT omentin-1 (A) mRNA expression, (B) protein expression, and (C) plasma levels in obese groups and lean controls. Omentin-1 mRNA expression is significantly decreased in the VAT of all obese groups as compared to the lean controls (N = 60). Representative blot and densitometric analysis of omentin-1 normalized to α-tubulin revealed that protein expression is also significantly decreased in the VAT of all obese groups as compared to the lean controls (n = 3 per group). For plasma levels, values presented are the mean ± SD of individual patients (N = 72). Group comparison by Kruskal-Wallis and post hoc Dunn's test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

PO6-19-YI
Targeting extracellular RNA mitigates hepatic lipotoxicity

Archana Tewari[1], Rohit Sinha[1]
[1]*Sanjay gandhi posgtraduate institute of medical sciences, Endocrinology, Lucknow, India*
Email: anthony.rohit@gmail.com

Background and aims: Non-alcohol related steatohepatitis (NASH) is a clinically serious stage of non-alcohol related fatty liver disease (NAFLD). Histologically characterized by hepatocyte ballooning, immune cell infiltration and fibrosis, NASH at a molecular level involves lipid induced hepatocyte death and cytokine production. Currently, there are very few diagnostic biomarkers available to screen NASH, and no pharmacological intervention is available for its treatment. Therefore, this work aimed to study the effect of extracellular RNA (eRNA) in modulating apoptosis and inflammatory response in human hepatocytes through cell culture model and to test the efficacy of eRNA inhibition via ribonuclease 1 (RNase 1) administration to limit NASH progression in mouse model.

Method: Cell culture model of NASH was established using palmitic acid (PA) treated HepG2 cells. Levels of eRNA in the conditioned media from PA treated HepG2 cells were assessed using kit based Quantifluor detection assay. Cell viability was assessed through 3- (4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Lipid droplets in HepG2 cells were assayed by Oil Red O Stain. Effect of RNase 1 on apoptotic and proinflammatory signalling pathways induced by eRNA in hepatocytes was analysed using western blotting, quantitative real time polymerase chain reaction (qRTPCR) and immunofluorescence techniques. Effect of eRNA in mitochondrial dysfunction was studied using JC1 staining method and Mitosox red assay. We also determined the pharmacological efficacy of RNase 1 in reducing NASH pathogenesis in a pre-clinical mouse model where C57BL/6N mice were fed high fat methionine choline deficient diet (HFMCD) diet for 4 weeks. Intra-peritoneal RNase 1 injections were given to another group of animals which were on HFMCD diet for 2 weeks. RNase 1 treatment was given every alternate day for 2 weeks. The total duration of experiment was for 4 weeks. After sacrifice, liver histology was assessed by Haematoxylin and Eosin (HandE) staining. Levels of serum alanine transaminase (ALT), and liver triglyceride (TG) were estimated by commercially available kits. Proinflammatory signalling pathways were analysed using western blotting, quantitative real time PCR and immunofluorescence techniques.

Results: In this study, we show that hepatocyte damage by lipotoxicity results in the release of extracellular RNAs (eRNAs) which serve as damage-associated molecular patterns (DAMPs) that stimulate the expression of pro-apoptotic and pro-inflammatory cytokines, aggravating inflammation, and cell death in HepG2 cells. Furthermore, the inhibition of eRNA activity by RNase 1 significantly increased cellular viability and reduced NF-kB mediated cytokine production. Similarly, RNase 1 administration significantly improved hepatic steatosis, inflammatory and injury markers in a murine NASH model. In summary, our results put forward eRNA released by injured hepatocytes under a lipotoxic environment constituting a feed forward loop, wherein hepatocyte injury is intertwined with the maintenance of a pro-inflammatory environment.

Conclusion: This study, for the first time, underscores the therapeutic potential of inhibiting eRNA action as a novel strategy for NASH treatment. Our results provide an insight into the pathological role of eRNA released by injured hepatocytes and put forth a proof-of-concept for limiting eRNA action to blunt NASH progression.

PO7-03-YI
Disruption of hepatic integrity and lipid composition by hypercaloric diets in steatotic liver disease

Maria Joao Meneses[1 2], Barbara Patrício[3], Getachew Debas Belew[4], Fabrizia Carli[3], John Jones[4], Amalia Gastaldelli[3], Maria-Paula Macedo[1 5]
[1]NOVA Medical School, NOVA University of Lisbon, Lisbon, Portugal, *[2]DECSIS II Iberia, Lda, Évora, Portugal*, *[3]National Research Council (CNR), Institute of Clinical Physiology (IFC), Pisa, Italy*, *[4]CNC-Center for Neuroscience and Cell Biology, Coimbra, Portugal*, *[5]APDP-Diabetes Portugal, Education and Research Center, Lisbon, Portugal*
Email: mariajoaocmo@gmail.com

Background and aims: Steatotic liver disease (SLD) has been linked to the consumption of lipid- and carbohydrate-rich diets. These dietary patterns have also been associated with dysbiosis, dysmetabolism, and increased intestinal permeability. Lipids are crucial for the structure of cell membranes, while tight junctions play a pivotal role in preserving membrane integrity and controlling permeability. Additionally, leaky gut has been identified as a risk factor for the development of SLD. However, the impact of these diets on hepatic tight junction in the context of SLD is still to be unveiled. Herein, we hypothesize that high-fat and/or high-carbohydrate diets may disrupt tight junctions in both the intestine and the liver, by impacting key proteins and lipids necessary for maintaining their integrity and association with SLD progression.

Method: Male C57BL/6J mice were fed four different diets for 18 weeks: standard chow (CTR; n = 12), high-fat chow (HF; n = 12), and the previous diets with water enriched with 30% sugar (55% fructose/45% glucose; HS and HFHS, respectively; n = 11). The night before being euthanized, mice were injected with deuterated water to assess lipid fluxomics. Histological analysis of the intestinal and liver tissues was performed, and the levels of tight junctions' proteins were evaluated. Additionally, considering the significance of lipids in the development of SLD and cell membrane composition, lipidomics of the liver tissue was evaluated.

Results: All experimental groups except CTR developed SLD. There was decreased hepatic expression of occludin in the HS and HFHS animals and tricellulin in the HFHS group. The latter also presented increased *de novo* synthesis of ceramides (e.g. Cer (d18:1/16:0), Cer (d18:1/20:0), and Cer (d18:1/24:1)) compared to the CTR ($p < 0.0001$) and both HFHS and HF had decreased hepatic phosphatidylcholines levels (PC34:1, PC34:2), associated with decreased PC synthesis. However, in the HF group, no changes in hepatic tight junctions' proteins were observed. Surprisingly, in the intestine, exposure of the animals to HF, HS and HFHS diets for 18 weeks did not lead to changes in jejunum, ileum or colon' tight junctions.

Conclusion: These findings suggest that changes in liver tissue permeability occur through disruption of occlusion junctions. Moreover, these changes occur prior to alterations in intestinal permeability. Importantly, consumption of diets rich in sucrose or sucrose and lipids are linked to hepatic steatosis and hepatic tight junction disruption.
Acknowledgements: EU Horizon 2020, under Marie Sklodowska-Curie Grant Actions, Agreements N. 722619 and 734719 and EU Horizon Europe, agreement n. 101080329 (PAS GRAS).

PO7-07-YI

Quantitative assessment of liver steatosis in patients with metabolic dysfunction-associated steatotic liver disease (MASLD) based on plasma extracellular vesicles in correlation with ultrasound attenuation parameter (UAP)

Eleni-Myrto Trifylli[1 2 3], Anastasios Kriebardis[1], Nikolaos Papadopoulos[4], Charikleia Kranidioti[3], Evangelos Koustas[2], Sofia Vasileiadi[3], Sotirios Fortis[1], Effie Papageorgiou[1], Aloizos Georgios[2], Ioannis-Georgios Koskinas[3], Spilios Manolakopoulos[3], Melanie Deutsch[3]
[1]University of West Attica (UniWA), Laboratory of Reliability and Quality Control in Laboratory Hematology (HemQcR), Department of Biomedical Sciences, Section of Medical Laboratories, School of Health and Caring Sciences, Egaleo, Greece, *[2]417 Army Share Fund Hospital, First Department of Internal Medicine, Athens, Greece*, *[3]National and Kapodistrian University of Athens, 2d Academic Department of Internal Medicine, Medical School, Hippokration General Hospital, Athens, Greece*, *[4]401 General Army Hospital of Athens, Second Department of Internal Medicine, Athens, Greece*
Email: trif.lena@gmail.com

Background and aims: Over the last decades a remarkable increase in chronic liver diseases related to Metabolic dysfunction-associated Steatotic Liver Disease (MASLD), has been reported worldwide, affecting 25-30% of the global adult population and constituting the second principal cause of liver transplantation in the USA and Europe. The increasing number of MASLD patients necessitates non-invasive methods for the assessment of hepatic steatosis such as imaging methods (e.g. ultrasound attenuation parameter (UAP) and/or serologic biomarkers. Among the latest extracellular vesicles (EVs) have gained a special interest nowadays. EVs constitute heterogeneous nanoparticles produced by several cells, including hepatocytes under lipotoxicity. Changes in the amount of secreted EVs have been closely related to the inflammatory reaction, the development of Non-Alcoholic Steatohepatitis (NASH), and fibrosis or Hepatocellular cancer. The aim of the present study is to evaluate EVs in MASLD patients and to correlate them with UAP as a quantitative assessment method of liver steatosis.

Method: We randomly selected non-diabetic patients with ultrasound findings of MASLD and transaminase levels up to 3 times higher than the normal range. We excluded patients with active infectious or inflammatory disease, other chronic liver diseases, and active malignancy, as well as with other causes of hypertransaminasemia. All the patients gave their consent for their participation and the handling of their biological specimens for the purposes of this study. Evaluation of steatosis via the UAP measurement was mediated via the performance of transient elastography by a trained operator, using the FT100 device by HISKY Med. Meanwhile, we performed quantification of plasma EVs via Nanoparticle tracking analysis technology, using the NanoSight NS300 device by Malvern Panalytical Ltd. The samples were diluted in particle-free Phosphate-buffered saline to an acceptable concentration, according to the manufacturer's recommendations and they were analyzed under constant flow and temperature conditions.

Results: We evaluated 26 patients with a mean age of 55 ± 11 years, with 46% of them being males. They presented a mean BMI of 27.36 ± 3.7 kg/m^2, UAP 251 ± 48.3 dB/m, ALT 64.2 ± 37.1 IU/L, and mean concentration of EV particles/ml 4.04e+12 ± 2.54e+13. We found that the presence of steatosis (UAP ≥244) was associated with increased plasma EV levels (figure1), while the severity of steatosis was proportional to the levels of vesicles with significantly increased levels in the cases with S3 steatosis grade (figure2). Finally, the ROC analysis of the measurement of the mean plasma vesicle concentration as a method of correlating steatosis diagnosis and UAP revealed a sensitivity of 92.3% and a specificity of 69.2%, p < 0, 001 with AUC 0.817 and a diagnostic limit of mean vesicle concentration above 247·10^6 particles/ml.

Conclusion: There is a correlation between the existence of steatosis and the presence of increased plasma EV levels in MASLD patients, while the severity of steatosis is proportional to the levels of vesicles with significantly increased levels S3 grade cases of steatosis and slightly different between subjects with S1 and S2 grade. A diagnostic cut-off value of mean vesicle concentration above 247·10^6 particles/ml has been revealed for the diagnosis of steatosis, with a statistically significant AUC of 0.817, overall sensitivity of 92.3%, and specificity of 69.2%.

Figure:

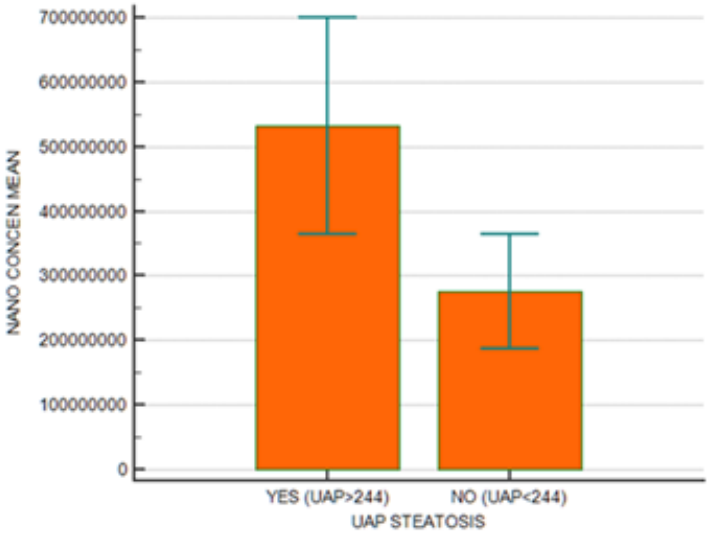


Figure 1. EVs concentration in the presence of steatosis

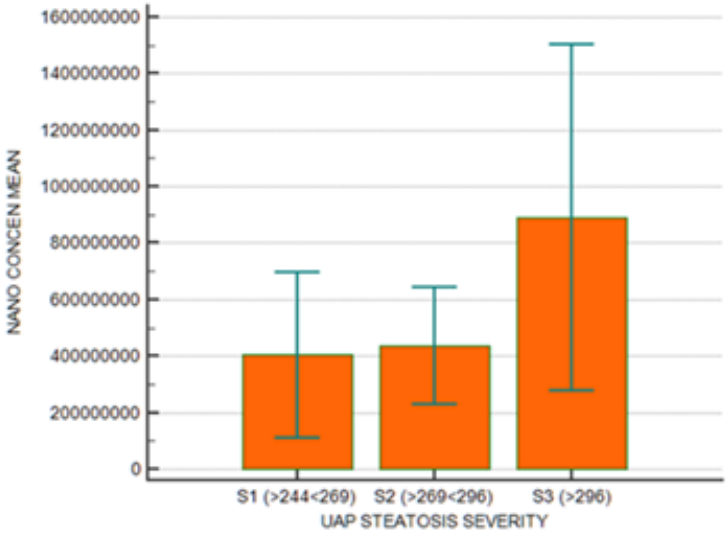


Figure 2. EVs concentration in steatosis severity

PO7-10
GCKIII kinases in hepatocellular lipotoxicity: role in NAFLD and beyond

Margit Mahlapuu[1], Emmelie Cansby[1], Mara Caputo[1], Hanns-Ulrich Marschall[2], Matthias Blüher[3]
[1]University of Gothenburg, Department of Chemistry and Molecular Biology, Gothenburg, Sweden, *[2]University of Gothenburg, Department of Molecular and Clinical Medicine/Wallenberg Laboratory, Institute of Medicine, Gothenburg, Sweden*, *[3]University of Leipzig, Department of Medicine, Leipzig, Germany*
Email: margit.mahlapuu@gu.se

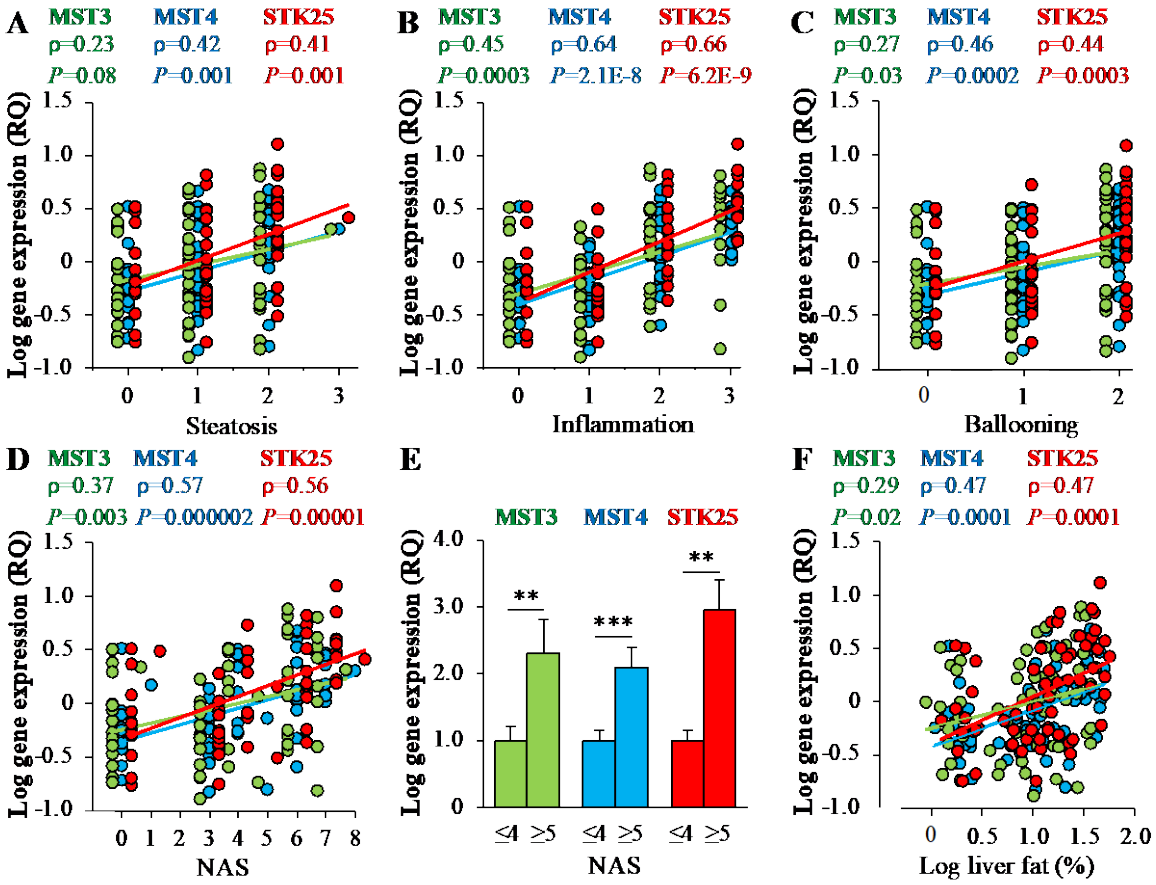
Background and aims: Non-alcoholic fatty liver disease (NAFLD) is defined by excessive accumulation of lipid droplets within hepatocytes. While significant advancement has been made toward characterizing the molecular pathogenesis of hepatic fat accumulation, as well as the aggravation of NAFLD into its severe form non-alcoholic steatohepatitis (NASH), much remains to be resolved. The aim of this project was to identify novel regulators of liver lipid metabolism and NAFLD/NASH susceptibility, and to decipher their mode-of-action.

Method: We have used translational studies in well-characterized patient cohorts, cultured human hepatocytes, and genetic mouse models to investigate the role of GCKIII kinases MST3, MST4, and STK25 in the regulation of hepatocellular lipotoxicity.

Results: First, we found that MST3, MST4, and STK25 kinases exhibit a highly specific subcellular distribution patter, being exclusively localized around lipid droplets in human and rodent hepatocytes. Furthermore, we detected a significant positive correlation between MST3, MST4, and STK25 transcript abundance in human liver biopsies and all three individual lesions of the NAFLD Activity Score (NAS) used for clinical diagnosis (i.e., histological scores of liver steatosis, lobular inflammation, and hepatocellular ballooning) as well as total NAS (Figure 1). Consistently, we observed that small interfering (si)RNA silencing of MST3, MST4, or STK25 in human hepatocytes markedly reduces intracellular lipid accumulation, which is mediated by increased β-oxidation and very low-density lipoprotein (VLDL)-TAG secretion (i.e., output), combined with decreased TAG synthesis (i.e., input). Finally, we found that, when challenged with a NASH-inducing diet, the livers from STK25 or MST3 knockout mice display substantial suppression of integral NASH features compared with wild-type livers: along with less steatosis, we observed attenuation of oxidative stress, inflammation, and nutritional fibrosis, combined with reduced hepatocellular damage.

Conclusion: Antagonizing hepatic GCKIII signaling appears to enable targeting of the initiating nexus for lipid-triggered liver injury in NAFLD, warranting further investigations in basic biology as well as clinical implications of these kinases.

Figure: Hepatic expression of GCKIII kinases is positively correlated with NAFLD severity. Correlation between mRNA levels in human liver biopsies and the individual histological lesions of NAS (A-C), the total NAS (D), and subjects with low vs. high NAS (E). (F) Correlation between mRNA levels and hepatic fat content measured by magnetic resonance spectroscopy.



PO7-11
Genetic evidence for a role of PON2 in liver fibrosis and generation of PON2 knockdown LX-2 cells

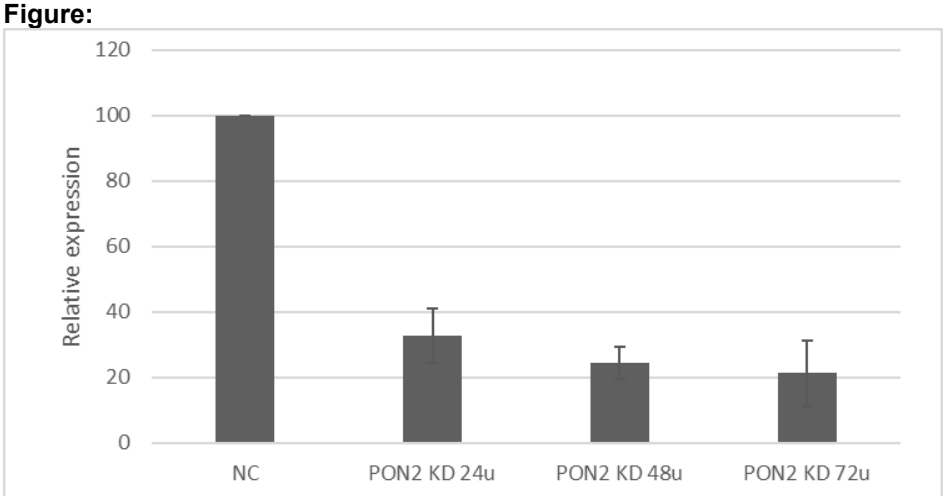
Tammy Huybrechts[1], Evelien Van Dijck[1], Ellen Steenackers[1], Erik Fransen[1], Sven Francque[2], Wim Van Hul[1]
[1]*University of Antwerp*, [2]*Antwerp University Hospital*
Email: tammy.huybrechts@uantwerpen.be

Background and aims: The *PON* gene family is a group of anti-oxidant enzymes consisting of 3 members: *PON1, PON2, PON3*. A role for oxidative stress in the metabolic dysfunction associated steatotic liver disease (MASLD) pathogenesis, that was already described in literature, makes this family an interesting target for further investigation. We looked at the role of rare genetic variants on different MASLD related parameters and started functional studies to determine via which mechanisms this gene family has an influence on the disease outcome.

Methods and results: For this study we used the HEPADIP cohort, a cohort containing obese patients with or without the presence of MASLD. A detailed metabolic profile and DNA samples are available from these patients. We performed Next Generation Sequencing with single-molecule molecular inversion probes for the 3 genes of the *PON* family. To look for association of rare variants with the MASLD phenotype, four different gene-based test were used. The Combined and Multivariate Collapsing Method for Rare Variants (CMC), the Variable Thresholds methods test (VT) and the Kernel-Based Adaptive Cluster test (KBAC) are variant burden tests, which assume that all rare variants are deleterious. A c-alpha (C (α)) test was also employed to investigate the distribution of both beneficial and deleterious alleles. We looked for an association with steatosis, inflammation, lobular ballooning, fibrosis and MASLD staging score. An association between *PON2* and fibrosis was found (p values: CMC p = 0, 003; VT p = 0, 001; KBAC p = 0, 003; C (α) p = 0, 001).In the liver, hepatic stellate cells (HSC) are regulated by a complex network and could be activated by different stimuli (e.g. inflammation mediators). The activation of HSC leads to collagen production which can lead to fibrosis. Since this cell type is responsible for the formation of fibrosis, it is the preferred study model. Therefore we used the LX-2 cell line which is an immortalized HS cell line.To determine the role of *PON2* in the formation of fibrosis and more general the pathogenesis of MASLD, we targeted *PON2* with 10nM siRNA to generate a knockdown (KD). Therefore we used RNAiMAX lipofectamine to transfect the cells. As shown in figure 1, KD efficiency of 70% at RNA level was obtained. The *PON2* KD efficiency will also be validated at the protein level with Western blot. We will look at the impact of this KD on the expression levels of different fibrosis genes (*ACTA2, COL1A1, FN1*) with qPCR. In a later stage we will also add genes that are of importance in other stages of the disease e.g. inflammation, lipogenesis, and in addition RNA sequencing will be performed.

Conclusion: We generated genetic evidence for a role of PON2 in liver fibrosis and generated a valuable cellular model to assess the role of PON2 in fibrosis formation.

Figure:



Relative expression level of PON2 at different RNA collection points

PO7-12-YI
Glutamyl aminopeptidase A (APA): a novel secreted kidney protein impacting liver homeostasis

Sharleen Hamad[1], Anna Permyakova[1], Saja Baraghithy[1], Asaad Gammal[1], Yossi Tam[1]
[1]*The Hebrew University of Jerusalem, Jerusalem, Israel*
Email: Sharleen.hamad@mail.huji.ac.il

Background and aims: metabolic dysfunction-associated steatotic liver disease (MASLD) is a prevalent cause of chronic hepatic disorders worldwide, affecting over 25% of the global population and leading to significant morbidity and mortality. Glutamyl/Aspartyl aminopeptidase A (APA), encoded by the *Enpep* gene, is an enzyme primarily expressed in the renal system. It catalyzes the hydrolysis of angiotensin II into angiotensin III, thereby playing a role in central blood pressure regulation. Recent evidence indicates a significant upregulation (327%) of *Enpep* in a mouse model of MASLD induced by a high-fat diet (HFD). However, the specific involvement of APA/*Enpep* in hepatic steatosis and whole-body metabolism, beyond its established role in blood pressure modulation, remains unreported.

Method: We evaluated the expression and activity of APA in the serum, kidney, and liver of mice subjected to a 20-week HFD feeding, compared to mice on a standard diet (STD). Additionally, we quantified APA levels and activity in kidney and serum samples obtained from both lean and obese individuals. To investigate the effects of genetic deletion or pharmacological blockade of APA on metabolic profiles and liver homeostasis, we utilized CRISPR/Cas9 technology or administered a commercially available APA blocker (Amastatin; 3 mg/kg/day, ip), respectively, to male and female mice fed either STD or HFD for 20 weeks.

Results: We observed upregulated APA expression and activity in the kidney and liver of mice following HFD consumption. Furthermore, APA levels and activity were elevated in the circulation of both obese mice and humans. Positive correlations were found between APA levels/activity, BMI, and liver injury in humans. Global genetic deletion of APA in mice resulted in overall metabolic improvements and protection against HFD-induced hepatic steatosis and liver injury. Chronic pharmacological blockade of APA with Amastatin in obese WT mice led to multiple positive outcomes, including reduced body weight and fat mass, decreased total cholesterol levels, improved glucose tolerance and insulin sensitivity, increased total energy expenditure, and improved liver function with reduced steatosis

Conclusion: These findings provide compelling evidence for the metabolic impact of APA, derived from the kidney, on liver function in the context of obesity. The observed increase in kidney APA levels, followed by its release into the bloodstream, may contribute to the development of MASLD. Our findings suggest APA as a potential biomarker for the detection of MASLD. Furthermore, these findings shed light on the therapeutic potential of targeting APA with drugs for the treatment of obesity and MASLD.

PO7-14-YI
Cholesterol exacerbates microcirculatory abnomalities in non-alcoholic fatty liver disease

Evelyn Pereira[1], Beatriz Araujo[1], Carolina Martins[1], Karine Rodrigues[1], Raquel Silvares[1], Fernanda Guimarães[2], Edgar Flores[1], Patricia Silva[2], Anissa Daliry[1]
[1]Oswaldo Cruz Foundation, Laboratory of Clinical and Experimental Physiopathology, Rio de Janeiro, Brazil, *[2]Oswaldo Cruz Foundation, Laboratory of Inflammation, Rio de Janeiro, Brazil*
Email: evelyn_n_goulart@hotmail.com

Background and aims: Non-alcoholic steatohepatitis (NASH), the progressive inflammatory manifestation of non-alcoholic fatty liver disease (NAFLD) can lead to cirrhosis and liver failure. Studies demonstrate that cholesterol is an important lipotoxic molecule involved in the development and progression of NAFLD. In addition, studies show that microcirculatory changes are key components of NAFLD. Thus, in this study, we sought to investigate the role of cholesterol as an insult modulating the progression of microcirculatory damage in NAFLD and the underlying mechanisms.

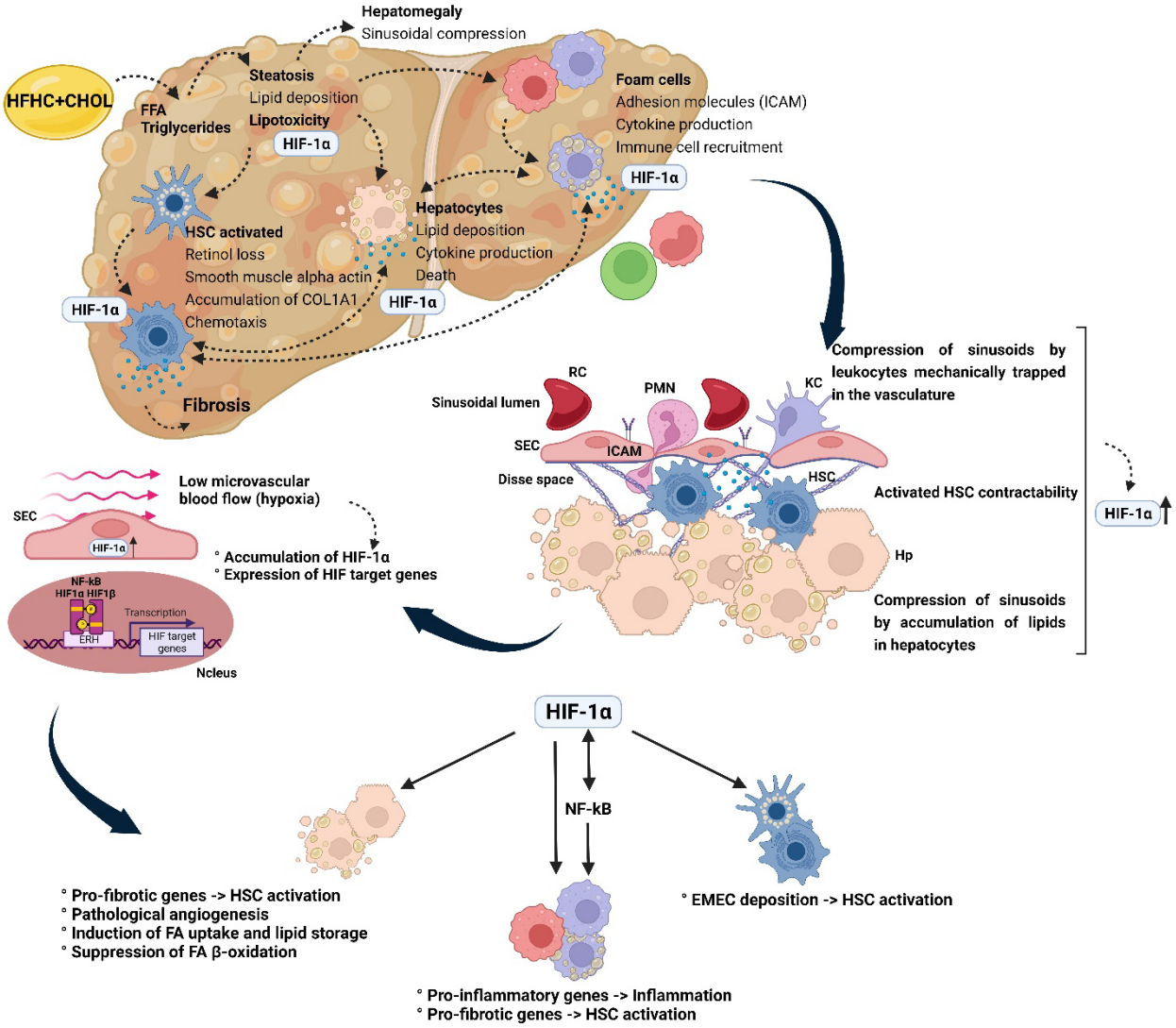
Method: First, the experimental model of NAFLD/NASH was induced in 20 male C57BL/6 mice by a high-fat, high-carbohydrate (HFHC) diet for 31 weeks. In addition, 10 mice received a 2% cholesterol-increased diet (HFHC + CHOL) between weeks 31 and 39 (CEUA L-012/2018 A2). Leukocyte recruitment and the number of activated hepatic stellate cells (HSC) in the microcirculation was assessed by intravital microscopy. Microcirculation blood perfusion was measured *in vivo* by laser speckle flowmetry. Results were expressed as mean ± standard deviation of the mean. Comparisons between groups were made using a one-tailed ANOVA, followed by Tukey's post hoc test. P values <0.05 were considered significant.

Results: At 39 weeks, the HFHC + CHOL group showed hepatomegaly (CTL: 0.06 ± 0.003 vs. HFHC + CHOL: 0.15 ± 0.009; grams; p <0.001) and liver with pale yellow discoloration, indicating hepatic steatosis. Histologically, the HFHC + CHOL group showed steatosis, both macrovesicular and microvesicular, liver inflammation, hepatocellular damage, and fibrosis compared with the other groups. The group receiving cholesterol showed stronger labelling for alpha-smooth muscle actin, increased transcription of COL1A1 in the liver (HFHC + CHOL: 40.8 ± 6.4 vs. CTL: 1.0 ± 0.1 and HFHC 14.2 ± 2.9; relative expression/β-actin; both p <0.001) and greater activation of HSC (HFHC + CHOL: 28.5 ± 3.9 vs CTL: 124.3 ± 3.0 and HFHC 56.5 ± 5.7; number of vitamin A+ cells; both p <0.001) compared to the other groups. Flow cytometry showed evidence of TH1 polarization by IFN-γ/IL -4 ratio in the HFHC + CHOL group (p = 0.022). The HFHC and HFHC + CHOL groups showed a 56% and 71% reduction in hepatic microvascular blood flow, respectively (both p <0.001), with greater impairment in the HFHC + CHOL group (p = 0.045). Cholesterol supplementation also resulted in higher leukocyte recruitment to the liver microcirculation, with greater rolling (HFHC + CHOL: 13.3 ± 1.4 vs. CTL: 2.2 ± 0.3 and HFHC 8.9 ± 0. 4; number of rolling leukocytes/30s; p <0.001 and p = 0.002, respectively) and leukocyte adherence (HFHC + CHOL: 13.0 ± 1.3 vs CTL: 2.0 ± 0.4 and HFHC 9.6 ± 1.0; number of adherent leukocytes/30s; p <0.001 and p = 0.023), in addition to increasing mRNA transcripts for HIF1A (HFHC + CHOL: 1.8 ± 0.14 vs CTL: 1.0 ± 0.12 and HFHC 1.4 ± 0.05; relative expression/β-actin; p <0.001 and p = 0.044) and ICAM-1 (HFHC + CHOL: 4.2 ± 0.1 vs CTL: 1.3 ± 0.4 and HFHC 2.2 ± 0.4; relative expression/β-actin; p <0.001 and p = 0.012, respectively) compared to the other groups.

Conclusion: We conclude that cholesterol acts worsening metabolic, hepatic, and inflammatory damage. In parallel, we have shown that cholesterol-induced hepatic microcirculatory abnormalities is at least in part, mediated by HIF1A transcription upregulation. Thus, chronic hypoxia may act as a factor that exacerbates cholesterol-induced lipotoxicity and, more importantly, may be a link between simple steatosis and NASH.

Figure:

Action of cholesterol as a lipotoxic agent in NAFLD.
Abbreviations: HFHC + CHOL: high-fat, high-carbohydrate diet plus 2% cholesterol; FFA: Free fatty acids; HSC: Hepatic stellate cell; Hp: Hepatocyte; RBC: Red blood cell; SEC: Sinusoidal endothelial cell; PMN Polymorphonuclear leukocytes; KC: Kupffer cell; ECM: extracellular matrix. Created by the author.



POSTER ABSTRACT PRESENTATIONS

CLINICAL SCIENCE

PO1-02-YI
The impact of artificial intelligence on the evaluation of fibrosis changes in steatotic liver diseases: a single center experience

Reha Akpinar[1], Camila De Carlo[1], Davide Panzeri[2], Nicola Pugliese[3], Vincenzo Belsito[1], Elena Pagani[2], Donato Inverso[4], Alessio Aghemo[1], Massimo Roncalli[1], Luigi Terracciano[1], Laura Sironi[2], Luca Di Tommaso[1]
[1]Humanitas University, Department of Biomedical Sciences, Milan, Italy, *[2]University of Milan-Bicocca, Department of Physics, Milan, Italy*, *[3]IRCCS Humanitas Research Hospital, Hepatology Unit, Milan, Italy*, *[4]IRCCS San Raffaele Scientific Institute, Division of Immunology, Transplantation and Infectious Diseases, Milan, Italy*
Email: luca.di_tommaso@hunimed.eu

Background and aims: In non-alcoholic steato-hepatitis (NASH) the risk of severe liver disease increases with the pathological stage of fibrosis. Accordingly, the fibrosis stage is the main end point for clinical trials on NASH patients. The assessment of fibrosis stage is a descriptive and semi-quantitative process, with only a moderate reproducibility even among expert liver pathologists. Moreover, it hardly illustrates a continuum of injury and thus has limited sensitivity to reflect changes in disease severity over time. Artificial Intelligence (AI) based approaches have been successfully applied to the evaluation of morphological features of NASH, including fibrosis. In particular, a quantitative assessment of collagen area (the ratio of collagen-stained pixels over full-biopsy-pixels) demonstrated superiority over semi-quantitative staging in predicting clinical decompensation.
Aim of this study is to test, in a series of NASH patients treated in a single center, the clinical impact of a AI-based approach in the extraction of quantitative features describing liver fibrosis.

Method: The series under study included 47 patients with a clinical and pathological diagnosis of NASH. Among these, 9 patients were enrolled in drug development clinical trials for NASH and underwent a post treatment biopsy. For each biopsy (n = 56) we obtained a Sirius Red digital slide (SR-WSI). Four pathologists with a different degree of experience evaluated slides for NASH pathological stage according to CNR system to calculate inter-observer agreement by Fleiss' kappa. Collagen was segmented from the whole slide images to extract several features including estimated collagen area (ECA) and entropy of collagen (EnC: compartmentalization of collagen-stained pixels).

Results: The overall agreement between pathologists on NASH stage was only moderate (κ: 0.45) with a substantial agreement for F3 and F4 cases (respectively κ of 0.63 and 0.73). AI-based approach highlighted a progressively and significantly increases of ECA from F2 to F3 to F4 (5.1% ± 0.6; 9.4% ± 0.9; 16.8% ± 1.5; p: 0.005). EnC showed similar trend (1.5 ± 0.4; 1.7 ± 0.5; 2.3 ± 0.3) with significant differences between F2/F4 and F3/F4 (p: 0.005). AI disclosed a higher level of heterogeneity of fibrosis in cases belonging to F3 pathological stage, with ECA and EnC ranging respectively between 3% and 22% and 0.9 and 3.1. A change in stage following medical treatment for NASH, was recognized in 33% by the pathologist as compared to 100% by AI (p: 0.009). Among cases without changes at microscopic level, two were characterized by a clear and homogenous decrease of ECA and EnC (false negative response according to pathological evaluation), while in the remaining cases, ECA and EnC were discrepant.

Conclusion: Our data confirm that AI-based methods allow to better identify the response to treatment with anti-NASH drugs, likely because AI characterizes the fibrosis process by its true, continuous, and non-categorical nature. It should also be noted that where the pathologist identifies a change in stage following therapy, quantitative findings are consistent with the microscopic diagnosis. Finally, there is a certain percentage of cases where the objective parameters generated by AI demonstrate only an initial process of modification of the fibrosis, undetectable to the human eye.

PO1-03
The effects of vitamin E and docosahexaenoic acid ethyl ester on non-alcoholic fatty liver disease (NAFLD)-a randomized double blind placebo controlled parallel group clinical trial (PUVENAFLD)

Naim Alkhouri[1], Raj Vuppalanchi[2], Anne-Cecile Bayne[3], Deanna McCarthy[3], Naga Chalasani[2]
[1]Arizona Liver Health, Hepatology, Chandler, United States, *[2]Indiana University School of Medicine, Gastroenterology and Hepatology*, *[3]DSM Nutritional Products*
Email: naim.alkhouri@gmail.com

Background and aims: Non-alcoholic fatty liver disease is the most common form of chronic liver disease with no approved treatment. Previous studies have demonstrated variable efficacy of omega-3 fatty acids and vitamin E. The primary objective of this trial was to determine the efficacy of the combination of vitamin E and omega-3 fatty acid (DHA EE) versus (vs) placebo on reducing liver fat content after 6 months of intervention in adults with NAFLD.

Method: This was a multi-center, randomized, double-blind, placebo-controlled, parallel 4-arm intervention superiority trial in adults with NAFLD. Patients were randomized to one of four treatment arms (vitamin E 1000 mg/daily + DHA 1.89 g/daily or combination arm, vitamin E 1000 mg alone, DHA 1.89 g alone, or placebo) following a 2:2:1:1 randomization stratified by Type 2 Diabetes (T2D) diagnosis. The primary objective of the trial was to determine the efficacy of DHA EE + vitamin E versus placebo in reducing hepatic fat fraction (%) relative to baseline after 6 months of intervention. Secondary objectives were to determine the effect of vitamin E or DHA EE alone versus placebo on reducing liver fat after 6 months of intervention. We also aimed to determine the change after 6 months of DHA EE and/or vitamin E intervention in anthropometric, metabolic, hepatologic, nutrient, and inflammatory parameters, and quality of life status.

Results: Our cohort consisted of 203 subjects with a mean age of 51 years, 53% female, 91% White, and 59% Hispanic. The primary objective of the trial was not met. Compared to Placebo, no statistically significant difference in the 6-month fat fraction were detected for Vitamin E + DHA EE ($p = 0.98$), DHA EE ($p = 0.14$), and Vitamin E ($p = 0.91$). Compared to Placebo, no statistically significant differences were detected in the 3-month or 6-month levels for ALT (U/L) or AST (U/L). No significant differences in CK18-M30 (a biomarker of hepatocellular death) or TNF-alpha (a biomarker of systemic inflammation) were detected for any of the active treatment arms. Compared to Placebo, no significant differences were detected for emotional well-being, energy/fatigue, general, pain, or physical functioning on the SF-36 questionnaire ($p > 0.05$ for all intervention group in comparison to placebo).

Conclusion: The combination of DHA EE + vitamin E or either agents alone did not demonstrate efficacy on reducing liver fat or other liver health parameters.

PO1-04
The long term safety and efficacy of Saroglitazar in metabolic dysfunction-associated steatohepatitis (MASH) related compensated cirrhosis: a prospective, single center real life experience

Sujit Chaudhuri[1], Agnibha Dutta[1]
[1]AMRI Hospitals, Salt Lake, Gastroenterology, Kolkata, India
Email: sourjya_c@yahoo.com

Background and aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a major global health problem which is one of the most common causes of cirrhosis worldwide. Many medicines are being evaluated for MASLD but none are approved for MASH (Metabolic dysfunction-associated steatohepatitis) related cirrhosis till date. Saroglitazar, a dual PPAR agonist has been approved in India for non-cirrhotic MASH. We tried to evaluate the safety and efficacy of Saroglitazar in MASH related compensated cirrhosis

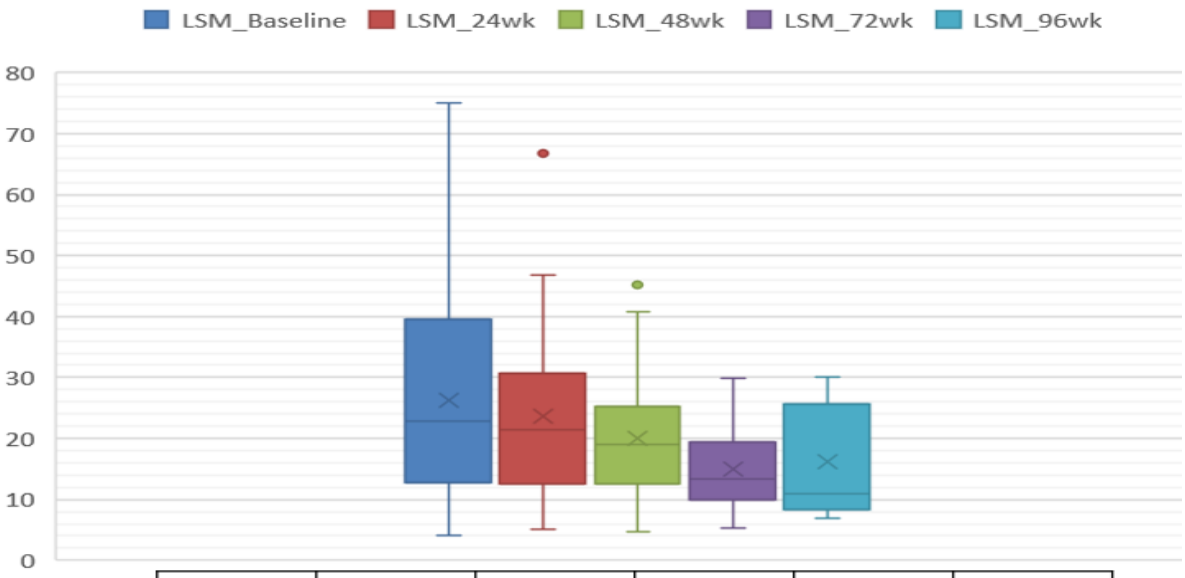
Method: A cohort of patients (n = 38, 60.5 % male, 53% diabetic, mean age 58.2±10.4 years and BMI 25.9±7.2kg/m^2) visited to outpatient department in Gastroenterology department of AMRI hospital (Salt lake, Kolkata, West Bengal, India) since August 2019 and diagnosed with MASH related cirrhosis without any sign and symptoms of decompensation on clinical, endoscopic and USG evaluation. They underwent elastography measurement with Echosens 503 Fibroscan® machine and treated with Saroglitazar 4mg once daily. The repeated measurement of steatosis (controlled attenuation parameter, CAP) and fibrosis (liver stiffness measurement, LSM) was performed at every 6 month. Wilcoxon signed rank test is applied for this non parametric data to find out the subsequent assessment at 24, 48, 96 and 144 weeks of follow-up, compared with the baseline. The safety and tolerability of drug is documented at each visit based on information shared by patient and their attendant and after evaluating the health related quality of life.

Results: The improvement at 96 week in fibrosis is 41.7 % (LSM decreased from median value 22.8 (25.8) to 13.3 (8.6) KPa, ($Z = -4.82$; $p < 0.001$)) and steatosis is 26.2 % (CAP reduced from median value 282 (84.5) to 208 (47) dB/m ($Z = -3.82$; $p < 0.001$)) compared to baseline. Out of 38 patients, 12 patients had follow-up at 144 weeks and shown continued treatment benefit with significant improvement in both fibrosis and steatosis by 38.9 % ($Z = -2.51$; $p = 0.01$) and 7.3 % ($Z = -2.24$; $p = 0.03$) respectively, compared from baseline. There was no therapy related side effects and discontinuation observed or reported during the entire treatment duration.

Conclusion: Saroglitazar is found to be effective in improvements of liver stiffness parameters for off label use in MASH related compensated cirrhosis. There is no evidence of any clinically significant liver injury and signs of any decompensation. Further studies are needed to confirm the efficacy and long-term safety of Saroglitazar.

Figure:

Fig: Box Whisker Plot showing Change of Liver stiffness with Time



LSM (kPa)	Baseline	24 Wk	48 Wk	96 Wk	144 Wk
n	38	38	38	38	12
Median (IQR)	22.8(25.8)	21.5(17.3)	19(11.5)	13.3(8.5)	10.9(16)
Z Score	-	-2.204	-3.606	-4.816	-2.510
P	-	.027	<.001	<.001	.012

PO1-05
Open data, explainable AI, data science and conventional NITs the recipe for new machine learning diagnostic tests on MASLD

Athanasios Angelakis[1 2 3]
[1]*Amsterdam Public Health Research Institute, Netherlands*, [2]*University of Amsterdam, Netherlands*, [3]*Amsterdam University Medical Centers, Netherlands*
Email: a.angelakis@amsterdamumc.nl

Background and aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) has a prevalence exceeding 25% in the general population. Non-invasive tests, namely the FIB-4 index, NFS, APRI, and AST/ALT, play a crucial role in differentiating advanced fibrosis stages (F012 vs. F34 in metavir score). Our objective entails developing and optimizing an interpretable machine learning model that employs the aforementioned non-invasive test parameters as features, surpassing their individual performances in diagnosing advanced fibrosis in MASLD patients.

Method: Open data from two cohorts, China (train) and Malaysia (test), are used, with 540 participants (early/advanced fibrosis: 391/149) and 147 participants (116/31) having liver biopsy-confirmed hepatic fibrosis. The datasets contain features like age, sex, BMI, Albumin (ALB), platelet (PLT), AST, ALT, ALT/AST ratio, AST/PLT ratio, presence of diabetes/impaired fasting glycemia (DM.IFG), FIB-4, NFS, and APRI scores. The machine learning model is trained, tuned, and validated using the train dataset, followed by testing on the test dataset. 10-fold cross-validation (10CV) enhances model robustness. Despite a relatively low train set size, categorical gradient boosted trees (catboost) are employed, exhibiting minimal overfitting and robustness in similar-sized tabular datasets. Feature engineering expands feature space by considering power and product combinations of initial features. Tuning the catboost model involves parameters iterators: 10, learning_rate: 0.21, and depth: 5, while other parameters retain default values. Shapley values from explainable AI are utilized to understand feature importance in catboost predictions.

Results: We conducted a comparative analysis between our catboost model (ML) and the four non-invasive tests (NITs) FIB-4, NFS, APRI, and AST/ALT using specific threshold values for advanced fibrosis, namely 1.30, -1.455, 0.64, and 0.87, respectively. The comparison was performed on identical folds within the 10-fold cross-validation (10CV) framework, with consistent random seed selection. The performance results of both the catboost model and the four NITs are illustrated in the figure below. Notably, our approach yielded significant enhancements in terms of the area under the curve (AUC), specificity, sensitivity, and F1-score, both in the train and test datasets, compared to the performance of FIB-4, NFS, APRI, and AST/ALT. By employing shapley values, we identified the three most influential features contributing to the predictability of our machine learning model. These features are FIB-4, NFS raised to the power of 6, and PLT raised to the power of 8.

Conclusion: By utilizing data science with catboost, feature engineering, and the parameters/scores from four NITs, we achieve superior performance and robustness compared to those NITs. Explainable artificial intelligence identifies the importance of FIB-4, NFS^6, and PLT^8, potentially leading to a novel non-invasive test. We propose a paradigm shift for classifying MASLD patients into early and advanced fibrosis stages. Linear cut-off values alone are inadequate, necessitating data science and explainable artificial intelligence to provide insights. Combining parameters from all four NITs in an interpretable machine learning framework improves outcomes.

Figure:

NIT	Spec. 10CV	Sens. 10CV	ROC-AUC 10CV	F1 10CV	Spec. Test	Sens. Test	ROC-AUC Test	F1 Test
FIB-4	0.8500+/-0.0744	0.4893+/-0.0855	0.6997+/-0.0692	0.5499+/-0.0797	0.9109	0.4783	0.7514	0.5714
NFS	0.8764+/-0.0549	0.4559+/-0.0893	0.7076+/-0.0678	0.5673+/-0.0802	0.9271	0.4706	0.7707	0.5854
APRI	0.8076+/-0.0600	0.4663+/-0.0945	0.6452+/-0.0538	0.4806+/-0.0792	0.8857	0.4524	0.7073	0.5205
AST/ALT	0.7483+/-0.0471	0.3521+/-0.1420	0.5445+/-0.0504	0.2994+/-0.1181	0.8140	0.3889	0.5655	0.2857
ML	**0.8845+/-0.0601**	**0.5903+/-0.0877**	**0.7671+/-0.0632**	**0.6425+/-0.0708**	**0.9434**	**0.6098**	**0.8343**	**0.6944**

PO1-06
Validation of HeparDx™ score (by Metadeq, Inc.) as a non-invasive test for the diagnosis of metabolic dysfunction-associated steatohepatitis (MASH)

Giulia Angelini[1], Silvia Sabatini[2], Erminia Lembo[1], Ornella Verrastro[1], Ann Daly[3], Olivier Govaere[3], Simon Cockel[3], Quentin Anstee[3], Amalia Gastaldelli[2], Geltrude Mingrone[1]
[1]Università Cattolica del Sacro Cuore, Translational medicine and surgery, Roma, Italy, *[2]National Research Council, Institute of Clinical Physiology, Italy*, *[3]University of Newcastle, Faculty of Medical Sciences, United Kingdom*
Email: giulia.angelini@unicatt.it

Background and aims: Metabolic dysfunction-associated steatohepatitis (MASH) is the most common liver disease worldwide and the leading cause of liver-related morbidity and mortality. The large number of MASH subjects with potential for progressive liver disease creates screening challenges because diagnosis of MASH currently relies on an invasive liver biopsy. Therefore, there is an urgent need to find non-invasive test (NITs) for MASH diagnosis and to monitor disease progression. Recently, we have developed a model to predict MASH or severe fibrosis that combines PLIN2 and RAB14 mean florescence intensity (MFI) in monocytes with waist circumference (WC), triglyceride (TG), alanine aminotransferase (ALT) and presence/absence of diabetes as covariates. Thus, we aim to assess the accuracy of a new score (HeparDx™ score) as a NIT for MASH with F≥2 in two randomized clinical trials with liver biopsies, i.e., the Bariatric Surgery Versus Non-alcoholic Steato-hepatitis-BRAVES trial and the Liquid Biopsy for NASH and Liver Fibrosis-LIBRA trials. Finally, we looked at the distribution of PLIN2 and RAB14 expression in liver biopsies from the European NAFLD registry.

Method: We analyzed data from two trials that included 198 subjects with histological-proven MASH (BRAVES cohort, n = 119) and noMASH (LIBRA cohort, n = 79). The new model was developed using the random forest algorithm that was trained in a randomly selected subgroup and validated in the remaining subjects of the two cohorts. Liver expression of PLIN2 and RAB14 was then investigated, by RNASeq, in liver biopsies of a separated cohort (European NAFLD Registry, n = 206).

Results: The subjects included in the study were 198, the mean BMI was 36.9 ± 10.2 kg/m^2 and diabetes prevalence was 36%. On histological examination, 35.6% of all participants had liver steatosis<5%, without inflammation and ballooning (noMASH); and 64.4% had MASH with F≥2. The new model using circulating PLIN2 and RAB14 together with ALT, GGT and HOMA-IR (HeparDx™ score) was able to discriminate subjects with MASH and F≥2 with AUROC 0.8 and accuracy of 74%. RNASeq of liver biopsies obtained from subjects enrolled in the European NAFLD Registry cohort showed that the highest PLIN2 expression was observed in MASH with F≥2 with a trend to decrease in fibrotic tissues (F3/F4) while RAB14 decreased significantly from F0/F1 to F2 to F3/F4.

Conclusion: Non-invasive tests that use peripheral blood monocyte as biomarkers are reliable for the diagnosis of MASH with F≥2. The HeparDx™ score have the potential to replace invasive liver biopsy-based histology for the diagnosis and can help in identifying patients eligible for MASH pharmacotherapy or surgery in clinical trials.

PO1-07-YI
Poor diet quality is associated with higher liver stiffness and higher cardiovascular risk in a cohort of Italian MASLD (metabolic dysfunction associated steatotic liver disease) outpatients

Rusi Chen[1], Chiara Abbati[2], Roberta Capelli[2], Ernestina Santangeli[2], Bernardo Stefanini[2], Simona Leoni[3], Fabio Piscaglia[2], Silvia Ferri[3]
[1]University of Bologna, Alma Mater Studiorum, Bologna, Italy, *[1]University of Bologna, Alma Mater Studiorum, Bologna, Italy*, *[3]Division of Internal Medicine, Hepatobiliary and Immuno-allergic diseases, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Policlinico di S'Orsola. Department of Medical and Surgical Sciences, Bologna*
Email: rusi.chen@gmail.com

Background and aims: Poor diet quality accounts for 11 million deaths and approximately 50 % of cardiovascular (CV) disease related deaths globally. Practical diet screening tools like REAP-S (Rapid Eating Assessment for Participants-Shortened Version) have been developed to quickly assess the diet quality in clinical setting. The aim of our study was to compare the physical, laboratory and ultrasound features and CV risk of the MASLD patients of our liver clinic, based on their REAP-S score.

Method: We enrolled 65 subjects (49 % females, median age 62 years) followed in our MASLD outpatient clinic with no advanced chronic liver disease. All patients received a complete anamnestic, physical, laboratory and ultrasound evaluation. Their physical activity and dietary habits were assessed using validated questionnaires, respectively IPAQ (International Physical Activity Questionnaire) score and REAP-S score, the latter ranging from 13 to 39 points (with a higher score indicating a higher diet quality). Skeletal muscle function was evaluated using hand-grip strength. The CV risk was predicted using Italian National Institute of Health calculator "Progetto Cuore". According to a median REAP-S score of 31, we divided our cohort into 2 groups, namely "A" or poor diet quality (with score <31): 32 subjects and "B" or good diet quality (with score ≥31): 33 subjects.

Results: The two groups didn't show statistical differences in demographic and anthropometric data (age, sex, weight, height, BMI, waist circumference), nor in auto-reported physical activity or in hand-grip strength. Similar transaminases, lipid profile, renal function and total protein levels were found in the two groups. Compared to group A, in group B albumin levels were higher (4.39 g/dL vs. 4.19 g/dL; p = 0.01) and gamma-GT levels were lower (41 U/L vs. 60 U/L; p = 0.04). Despite no differences in NAFLD fibrosis score and FIB-4, liver stiffness evaluated with two-dimensional shear wave elastography showed, though both still within normal range, higher values in group A compared to group B (6.7 kPa vs. 5.6 kPa; p = 0.01). Regarding CV risks factors, similar prevalence of smoking habits and diabetes mellitus were found in the two groups. Compared to group B, more patients in group A had been diagnosed and took medications for arterial hypertension (75 % vs. 36 %; p = 0.002) displaying a higher probability to experience a major cardiovascular event in the following 10 years (10 % vs. 5.8 %; p = 0.03).

Conclusion: In our cohort of MASLD outpatients, diet quality assessed with REAP-S was significantly associated to nutritional biomarkers like albumin and to laboratory and ultrasound findings linked to steatotic liver disease (gamma-GT, liver stiffness). Through an association to arterial hypertension, a poorer diet may also worsen overall cardiovascular risk. We therefore recommend special attention to diet quality in patients with MASLD.

PO1-10-YI
Persons diagnosed with metabolic dysfunction-associated steatotic liver disease are at an increased risk of severe depression in a Swedish nationwide cohort study

Hanne Åström[1], Axel Wester[1], Hannes Hagström[1 2]
[1]Karolinska Institutet, Department of Medicine, Huddinge, Stockholm, Sweden, *[2]Karolinska University Hospital, Dvision of Hepatology, Department of Upper GI, Stockholm, Sweden*
Email: hanne.astrom@ki.se

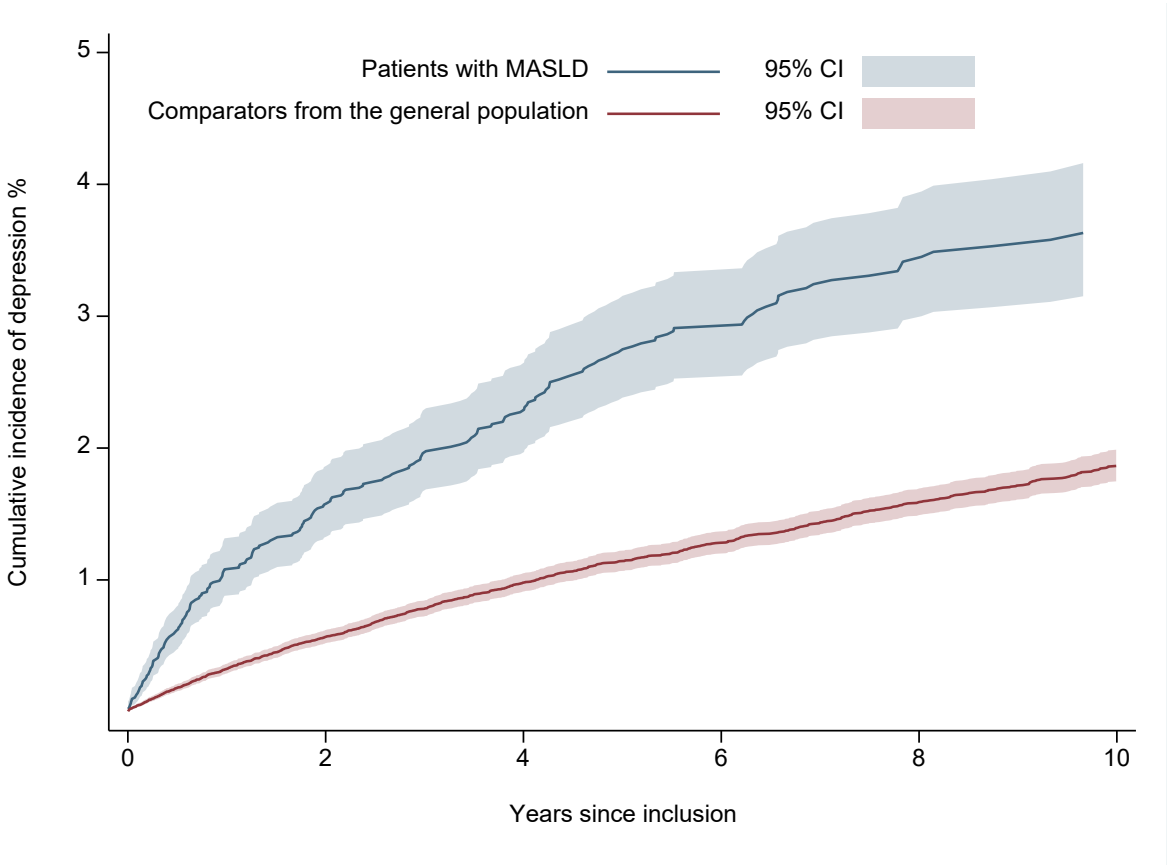
Background and aims: Previous research have suggested that depression is more common in persons with metabolic dysfunction-associated steatotic liver disease (MASLD), but it is unclear who is at greatest risk of developing severe depression. Here, we aimed to investigate the incidence rate of severe depression in a nationwide cohort of persons with MASLD.

Method: We identified all persons with administrative coding for MASLD/NAFLD in the Swedish National Patient Register 2006-2020 and matching was performed for age, sex and municipality with up to 10 comparators from the general population. The primary outcome was severe depression, defined as a depression requiring inpatient or outpatient psychiatric care. Logistic regression was used to calculate odds ratios (OR) for severe depression in MASLD at or before baseline. Cox regression was used to calculate hazard ratios (HR) of severe depression in MASLD, adjusted for cardiovascular disease, chronic kidney disease, hypertension, chronic obstructive pulmonary disease, diabetes, obesity, education level and marital status. The cumulative incidence of severe depression was calculated at 5 and 10 years, while accounting for competing risks (non-depression death).

Results: In total, we included 11, 301 persons with MASLD and 104, 205 comparators with a median follow-up time of 2.6 and 4.0 years, respectively. The median age at inclusion was 56 years. The aOR for severe depression at baseline was 1.4 (95% CI 1.2-1.5) and the aHR for incident severe depression was 1.8 (95% CI 1.5-2.1), compared to the general population comparators. The cumulative incidence of severe depression at 5 years was higher in MASLD than in comparators (2.7%, 95% CI 2.4-3.1 vs. 1.1%, 95% CI 1.1-2.0, respectively [Figure]). The aHR for severe depression was higher in men than women (men: 2.2, 95% CI 1.7-2.7 vs. women: 1.5 95% CI 1.2-1.9) and people of lower age (<50: 2.1 95% CI 1.7-2.5 vs. >65: 1.0, 95% CI 0.6-1.8).

Conclusion: Persons with MASLD had a 1.8-fold higher rate of severe depression requiring inpatient or outpatient psychiatric care compared to comparators from the general population. Some subgroups, such as men, seemed to have a slightly higher rate of severe depression possibly requiring closer monitoring for depression during follow-up. In addition, people of older age presented with a lower rate of severe depression than younger patients, possibly due to the competing risk of mortality.

Figure: Cumulative incidence of severe depression in patients with MASLD/NAFLD and comparators from the general population.



PO1-11-YI
Cytokine's level during the pregnancy with MASH and obesity under the developed complex therapy program

Lina Bahniy[1]
[1]I. Horbachevsky Ternopil National Medical University, Obstetrics and Gynecology №2, Ternopil, Ukraine
Email: bahnii@tdmu.edu.ua

Background and aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) is one of the most common diseases of the hepatobiliary system, and represents a wide spectrum of pathological conditions from simple steatosis with accumulation of lipids in the liver parenchyma with a fat content exceeding 5% of the liver volume to metabolic dysfunction-associated steatohepatitis (MASH), characterized by chronic inflammation. Our aim is the assessment the state of the cytokine profile in pregnant women with MASH against the background of varying degrees of obesity under the influence of the developed complex therapy program.

Method: We've examined 98 pregnant women with MASH in combination with obesity. All pregnant women were prescribed complex therapy including vitamin E at a dose of 400 IU/day, ursodeoxycholic acid (UDCA) at a dose of 15 mg/kg/day and L-carnitine at a dose of 3 g per day for three months continuously. To evaluate the cytokine profile, the level of IL-1β, IL-6, IL-10, and TNF-α was determined by ELISPOT.

Results: Analysis of the cytokine profile in women with MASH and obesity showed the presence of links of systemic inflammation in the examined groups. The appointment of complex treatment contributed to a decrease in the activity of the inflammatory response, which was manifested by an improvement in the levels of indicators of the cytokine link. Thus, the level of IL-1β in group IA until the end of pregnancy was not significantly different from the control and decreased by 1.16 times, in group IB-by 1.90 times, in group IC-decreased by 2.69 times ($p<0.001$). With additional complex therapy, the level of IL-6 decreased in group IA by 1.41 times and was not significantly higher than in healthy pregnant women, in group IB-by 1.87 times, only in group IC IL-6 decreased by 2.09 times and didn't reach control indicators ($p<0.001$). In patients of the IA group, the content of IL-10 in blood serum after the course of treatment increased by 1.12 times, in the IB group-by 1.19 times, in the IC group-by 1.52 times ($p<0.001$). Similarly, TNF-α content in all groups decreased by 1.48, 1.60, and 1.53 times, respectively, compared to the initial level ($p<0.001$).

Conclusion: Metabolic dysfunction-associated steatohepatitis during pregnancy is accompanied by significant changes in the cytokine profile. The appointment of complex therapy is effective in the treatment of pregnant women with MASH due to cumulative and potentiating effects, which made it possible to reduce the frequency of obstetric and perinatal complications.

Figure:

Parameter	Control group (n = 30)	MASH (n = 98)			
			Overweight IA group (n = 26)	Obesity I degree IB group (n = 48)	Obesity II-III degree IC group (n = 24)
IL-1β, pg/ml	2.42 ± 0.09	1	3.45 ± 0.11*	6.38 ± 0.16*	9.99 ± 0.53*
		2	2.96 ± 0.08**	3.35 ± 0.12*, **	3.71 ± 0.15*, **
IL-6, pg/ml	3.27 ± 0.16	1	4.98 ± 0.29*	8.57 ± 0.38*	10.77 ± 0.48*
		2	3.51 ± 0.15**	4.56 ± 0.18*, **	5.13 ± 0.26*, **
IL-10, pg/ml	9.69 ± 0.21	1	8.71 ± 0.44	7.46 ± 0.37*	5.38 ± 0.49*
		2	9.83 ± 0.32**	8.91 ± 0.24**	8.22 ± 0.36*, **
TNF-alpha, pg/ml	4.51 ± 0.28	1	7.59 ± 0.39*	13.03 ± 0.36*	16.17 ± 0.42*
		2	5.12 ± 0.23	8.14 ± 0.19*, **	10.54 ± 0.27*, **
Notes: 1, 2-parameters in pregnant women before and after treatment; *-probability of difference of factors in relation to the control group ($p < 0.001$); **-probability of difference of factors in relation to factors before treatment ($p < 0.001$).					

PO1-14-YI

Association of hepatic fibrosis and chronic kidney disease in a real world cohort of metabolic-dysfunction associated steatotic liver disease (MASLD); implications for screening in an intelligent LFT (iLFT) platform

Marc Gurun[1], Paul Brennan[1 1], Damien Leith[1], Sava Handjiev[2], Aseil Khatib[3], John Dillon[1], Christopher Byrne[1]
[1]University of Dundee, Division of Molecular and Clinical Medicine, Dundee, United Kingdom, *[2]NHS Tayside, Dept of Biochemistry, Dundee, United Kingdom*, *[3]NHS Tayside, Dept of Gastroenterology, Dundee, United Kingdom*
Email: paul.brennan5@nhs.scot

Background and aims: The association between metabolic dysfunction associated steatotic liver disease (MASLD) and increased risk of chronic kidney disease (CKD) is well described, however, the contribution of hepatic fibrosis to the relative risk remains underexplored. In this study, we aimed to establish whether hepatic fibrosis is associated with increased CKD risk, descriptively explore differences in mortality, and assess cost implications of urea and electrolyte (UandE) screening, in a real-world MASLD cohort.

Method: A retrospective, observational study of patients who underwent intelligent liver function testing (iLFT) in Tayside, Scotland. Data was collected from routine healthcare records. We used descriptive methods to illustrate differences in mortality, and log-binomial models to quantify the risk of CKD and explore multifactorial risk. Finally, a cost-per-positive-screen cost analysis was explored

Results: In our cohort (n = 2, 046), 1, 418 (69.3%) persons had MASLD without fibrosis, and 628 (30.7%) had MASLD with fibrosis; with 155 (10.9%) and 123 (19.6%) respectively, with CKD. Among 2, 024 people, fibrosis (n = 623;19% with CKD) was associated with increased CKD risk (*a*RR = 1.33, CI 1.06-1.66, p = 0.016), adjusted for confounders. Among those with fibrosis and CKD, 39 (31.7%) deaths were observed, compared to 20 (12.9%) among those with CKD without fibrosis. Estimated cost of iLFT UandE testing for those with MASLD fibrosis was £9.45 per positive CKD screen.

Conclusion: Liver fibrosis is an independent CKD risk factor in this cohort of patients with MASLD. A higher proportion of people with both fibrosis and CKD died, and the cost of UandE screening per CKD case identified was low. Due to increased CKD risk among people with MASLD fibrosis, appropriate renal function screening should be considered within surveillance programmes.

Figure:

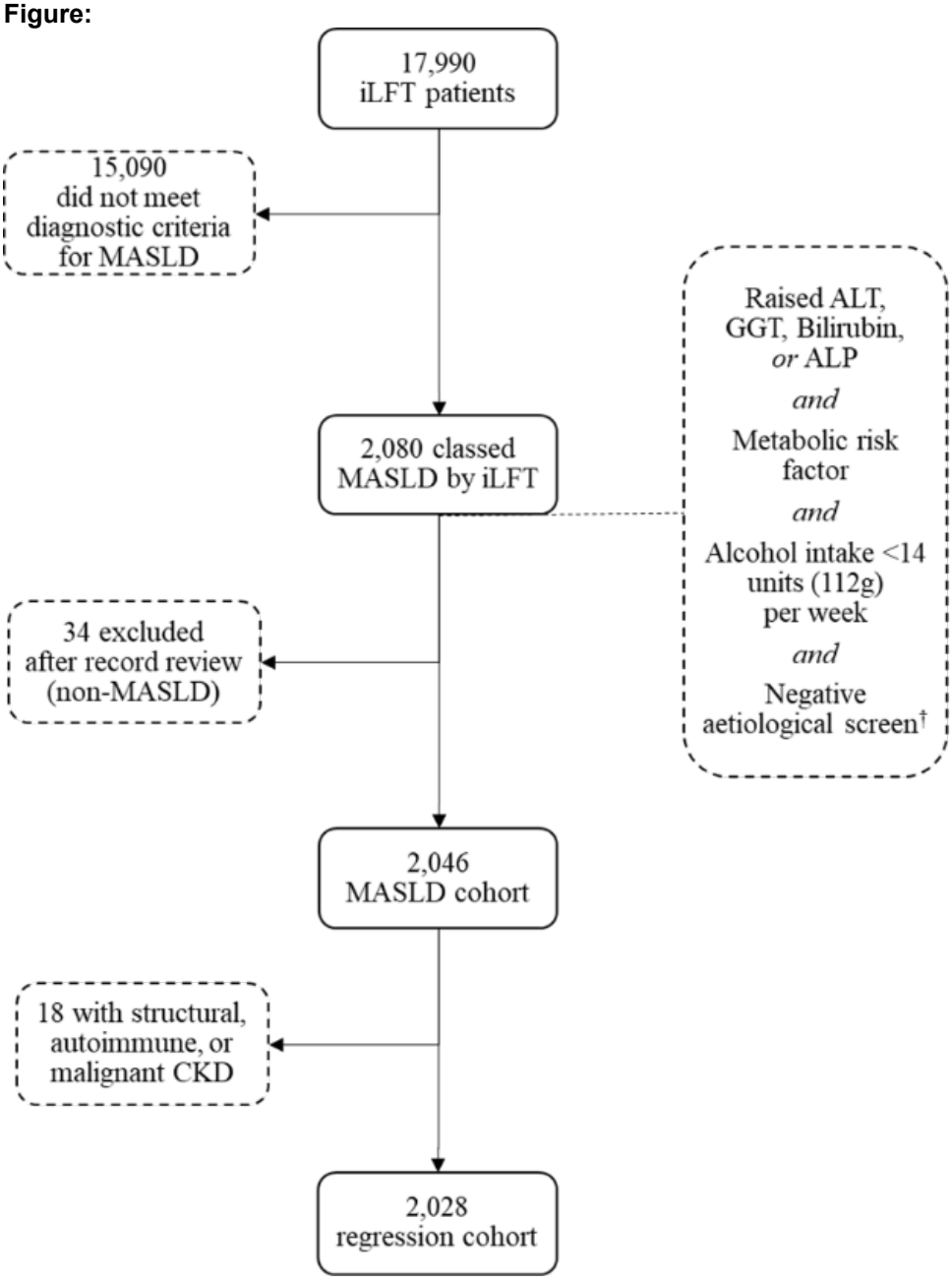


Figure 1: Participant flow diagram.

Abbreviations: iLFT, intelligent liver function testing platform; MASLD, metabolic dysfunction associated steatotic liver disease; ALT, alanine transaminase; GGT, gamma-glutamyltransferase; ALP, alkaline phosphatase; CKD, chronic kidney disease.
†Alpha-1 Antitrypsin, Wilson's Disease, Haemochromatosis, haemolysis, viral hepatitis, auto-immune hepatitis, primary biliary cholangitis, systemic lupus erythematosus, thrombocytosis.

PO1-18-YI
Improvement of HRQoL features and liver status after a 6-month dietary intervention in patients with metabolic dysfunction-associated steatotic liver disease

Gabriele Castelnuovo[1], Nuria Pérez Diaz del Campo[1], Chiara Rosso[1], Matteo Papurello[1], Gian Paolo Caviglia[1], Eleonora Dileo[1], Marta Guariglia[1], Irene Poggiolini[1], Angelo Armandi[1 2], Giorgio Maria Saracco[1 3], Simona Bo[1], Elisabetta Bugianesi[1 3]
[1]University of Turin, Department of Medical Sciences, Turin, Italy, *[2]University Medical Center of the Johannes Gutenberg-University, Metabolic Liver Disease Research Program, I. Department of Medicine, Mainz, Germany*, *[3]Città della Salute e della Scienza—Molinette Hospital, Gastroenterology Unit, Turin, Italy*
Email: gabriele.castelnuovo@unito.it

Background and aims: There is strong scientific evidence regarding the benefits of weight loss and exercise on metabolic dysfunction-associated steatotic liver disease (MASLD) and general quality of life (QoL). The aim of this study was to evaluate the impact of 6-month dietary intervention on QoL and liver status.

Method: A total of 60 persons with a diagnosis of MASLD according to the new nomenclature, were randomized to either a 6-month Mediterranean (MeD) or low-carbohydrate (LcD) diet, both with calorie restriction, or to a control diet (CD) without calorie restriction. The MeD consisted of 50-60% carbohydrate, 15% protein, 25-35% fat, while the LcD consisted of 35-40% carbohydrate, 25-30% protein, 30-35% fat. The CD group received general recommendations for lifestyle modifications, similar to those given in clinical practice. At baseline and after 6 months, liver steatosis was assessed with the controlled attenuation parameter (CAP) (Fibroscan®530, Echosens), and overall QoL was evaluated with the short form survey 36 (SF-36).

Results: The median age was 52 (range 42.5;61) years; 66.6% of participants were male, 16.6% had diabetes and the median Body Mass Index (BMI) at baseline was 30.28 Kg/m^2. After the 6-month intervention, the three diets induced a reduction in BMI ($p < 0.001$), waist circumference ($p < 0.001$), neck circumference ($p < 0.001$), diastolic blood pressure ($p = 0.003$) and insulin levels ($p = 0.028$). In general, CAP values were significantly lower after dietary treatment (299.1 dB/m vs. 273.5 dB/m, $p < 0.001$), but only LcD and CD groups led to a significant reduction in CAP levels (297.5 dB/m vs. 266 dB/m, $p = 0.002$ and 304.6 dB/m vs. 273.3 dB/m, $p = 0.003$, respectively), whereas no significant difference was found in the MeD group. Furthermore, at 6-month the three dietary approaches improved the health change outcome (MeD +30.3%, p = 0.028; LcD +29.2%, $p = 0.009$ and CD +40%, $p = 0.006$). However, only LcD significantly enhanced physical functioning (+5.5%, $p = 0.016$), while only MeD improved bodily pain (-5.55%, $p = 0.031$). At multivariate stepwise linear regression analysis a lower improvement in health change was associated with higher baseline CAP levels ($\beta = -0.18$, 95% CI -0.3;-0.01, $p = 0.038$) and sex ($\beta = -15.8$, 95% CI -30.0;-1.7, $p = 0.028$).

Conclusion: A 6-month nutritional intervention diet is associated with a reduction in CAP levels and an improvement in health change. Our results suggest that a personalized dietary approach could be integrated in order to improve the general health status of patients with MASLD.
This research has been supported by the Italian MIUR under the programme "Dipartimenti di Eccellenza 2018-2022", project code D15D18000410001 and by the University of Torino, grant number ROSC_RILO_21_01.

PO1-19-YI

From old to new: assessing the accuracy of non-invasive tests of fibrosis in patients with metabolic associated fatty liver disease and excessive alcohol consumption according to the recent definition

Annalisa Cespiati[1 2], Rosa Lombardi[1 2], Daniel Smith[1 2], Sofia Carvalhana[3], Cristina Bertelli[1], Giuseppina Pisano[1], Felice Cinque[1 2], Helena Cortez-Pinto[3], Anna Ludovica Fracanzani[1 2]
[1]SC Medicina ad Indirizzo Metabolico, Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy, Milan, Italy, *[2]Department of Pathophysiology and Transplantation, University of Milan, Italy*, *[3]Departamento de Gastroenterologia, Centro Hospitalar Universitário Lisboa Norte, Departamento de Dietética e Nutrição, Lisbon, Centro Hospitalar Universitário Lisboa Norte Portugal*
Email: annalisa.cespiati@unimi.it

Background and aims: Recently the nomenclature of non-alcoholic liver disease (NAFLD) has been replaced by steatotic liver disease, comprising different entities among which the metabolic dysfunction-associated steatotic liver disease (MASLD), characterized by liver steatosis and cardiometabolic risk factors, and met-ALD, which add to MASLD an excessive alcohol consumption. Due to the limitations of biopsy, non-invasive tests of fibrosis (NITs) (FIB4 and NFS) and liver stiffness measurement (LSM) by Fibroscan are used to diagnose fibrosis in NAFLD, but their performance in patients with metabolic comorbidities and excessive alcohol consumption is undefined. Aims 1) evaluate the diagnostic accuracy of NITs in MASLD and MetALD 2) evaluate their performance specifically in obese and diabetic.

Method: We enrolled 207 patients with biopsy proven steatosis, 185 MASLD (mean age 55 ± 12 ys, 60% males) and 22 MetALD (mean age 58 ± 10 ys, 82% males). Clinical, laboratory and Fibroscan data were collected within 6 months from biopsy. FIB-4<1.3, NFS<-1.455 and LSM<8 kPa ruled out advanced fibrosis (≥F3), FIB-4>3.25, NFS>0.675 and LSM≥8 kPa suggested advanced fibrosis.

Results: Fibrosis ≥F3 was 24% at histology in MASLD and 36% in MetALD. In MASLD, FIB-4 and NFS diagnosed ≥F3 in 6% and 7% and had indeterminate values in 42% and 45%, respectively. In MetALD FIB-4 identified ≥F3 in 18% and NFS in 9%, with indeterminate values in 64% and 54%, respectively. LSM ≥8 kPa in 58% of MASLD, and in 64% of MetALD. Both in MASLD and MetALD all NITs showed a lower accuracy in diagnosing (AUROCs in MASLD FIB-4 0.62; NFS 0.57; LSM 0.73; AUROCs in MetALD FIB-4 0.65; NFS 0.53; LSM 0.69) or excluding (AUROCs in MASLD FIB-4 0.75; NFS 0.73; LSM 0.73; AUROCs in MetALD FIB-4 0.64, NFS 0.78, LSM 0.69) advanced fibrosis compared to those reported in literature for NAFLD. 57% of the cohort was obese, 43% diabetic. For ruling-in advanced fibrosis, FIB-4 was the NIT with the worst performance both in obese (AUROCs obese vs non-obese 0.59 vs 0.69) and diabetic (AUROCs diabetic vs non-diabetic 0.58 vs 0.69), followed by LSM (AUROCs 0.68 vs 0.72 in obese vs non-obese and 0.70 vs 0.74 in diabetic vs non-diabetic). As for NFS, we found no difference of performance in obese vs non obese (AUROCs 0.57 vs 0.56), with a higher accuracy in diabetic vs non-diabetic (AUROCs 0.59 vs 0.50). For the exclusion of fibrosis, all NITs performed worse in obese vs non-obese (AUROCs FIB-4 0.72 vs 0.76; NFS 0.69 vs 0.81; LSM 0.70 vs 0.74) and in diabetic vs non-diabetic (AUROCs FIB-4 0.76 vs 0.71; NFS 0.63 vs 0.78; LSM 0.68 vs 0.72).

Conclusion: In patients with cardiometabolic risk factors, especially diabetes and obesity, and with excessive alcohol consumption either FIB4 or NFS or Fibroscan performed worse compared to NAFLD, with the latter having the higher accuracy. In MASLD and MetALD more accurate NITs should be developed to better identify patients with advanced fibrosis.

PO2-01
Low implementation rates of the American Gastroenterology Association (AGA) clinical care pathway in gastroenterology practice

Arjun Brahmbatt[1], Nina Pollard[1], Navneet Singh[1], Jessica Angell[1], Nicholas Haddad[1], John Haddad[1], Mousab Tabbaa[1], Naim Alkhouri[2]
[1]*Clinical Research Institute of Ohio*, [2]*Arizona Liver Health, Hepatology, Chandler, United States*
Email: naim.alkhouri@gmail.com

Background and aims: The AGA has released a clinical care pathway in 2021 to screen for NAFLD and identify NASH patients with at least stage 2 fibrosis (at-risk NASH). The pathway starts by screening for NAFLD in subjects with type 2 diabetes (T2DM) and those with 2 risk factors for the metabolic syndrome (MetS) then implements sequential testing with FIB4 and vibration controlled transient elastography (VCTE) to identify at-risk NASH. The aim of this study was to evaluate the implementation rate of this pathway in a community gastroenterology practice in the U.S. with access to VCTE within the practice.

Method: Consecutive patients that were seen in the gastroenterology practice for any indication over a period of 2 months were included. Demographic, laboratory, and imaging data were collected. Patients with T2DM or 2 MetS risk factors were considered eligible for the AGA pathway. Those with FIB4 >1.3 were considered eligible for VCTE. Descriptive statistics were performed using Microsoft Excel.

Results: 532 patients were included with a mean age of 59.3 years, mean BMI of 29.4 kg/m2, and mean HbA1C of 6.2%. 88 were diagnosed with T2D and 158 patients with 2 MetS risk factors [obesity (BMI >30 kg/m2), low HDL <40 mg/dL in men and <50 in women, high TG >150 mg/dL, or hypertension]. Of those that were diagnosed with T2DM, the mean FIB4 score was 1.83 and 42/88 (47.7%) had a FIB4 >1.3 indicating that they should have been referred for VCTE. Only 8/42 (19%) of eligible patients had a VCTE documented in their chart. Of those with 2 MetS risk factors, the mean FIB4 was 1.57 and 62/158 (39.2%) had FIB >1.3 making them eligible for VCTE; however, only 13/62 had a VCTE documented in their chart.

Conclusion: In a community-based gastroenterology practice with access to VCTE, a minority of patients that would be eligible for VCTE based on FIB4 >1.3 received the test. Increasing the implementation rates of the AGA pathway in gastroenterology practices will improve risk stratification of patients with NAFLD.

PO2-04-YI

The effect of metabolic dysfunction-associated steatotic liver disease on liver fibrosis progression and regression in virus-related liver disease: a multicenter longitudinal study

Felice Cinque[1 2], Jovana Milic[3], Stefano Renzetti[4], Federico Motta[5], Bischoff Jenny[6], Dana Kablawi[1 7], Wesal Elgretli[8], Giulia Besutti[3 9], Giuseppe Mancini[3 9], Sara Esperti[3 9], Marianna Menozzi[3 9], Stefano Calza[10], Cristina Mussini[3 9], Valentina Menozzi[3 9], Maria Daria Di Trapani[3 9], Jürgen Rockstroh[6], Giovanni Guaraldi[3 9], Giada Sebastiani[1 2 8]

[1]McGill University Health Centre, Chronic Viral Illness Service, Division of Infectious Diseases, Montreal, Canada, *[2]McGill University Health Centre, Division of Gastroenterology and Hepatology, Montreal, Canada*, *[3]University of Modena and Reggio Emilia, Modena HIV Metabolic Clinic, Modena, Italy*, *[4]University of Brescia, Department of Medical-Surgical Specialties, Radiological Sciences and Public Health, Brescia, Italy*, *[5]University of Modena and Reggio Emilia, Department of Physics, Informatics and Mathematics, Modena, Italy*, *[6]University Hospital Bonn, Department of Internal Medicine I, Venusberg Campus 1, Bonn, Germany*, *[7]McGill University Health Centre, Division of Gastroenterology and Hepatology, Montral, Canada*, *[8]McGill University, Division of Experimental Medicine, Montreal, Canada*, *[9]University of Modena and Reggio Emilia, Infectious Diseases Clinic, Azienda Ospedaliero-Universitaria, Policlinico of Modena, Modena, Italy*, *[10]University of Brescia, Unit of Biostatistics and Bioinformatics, Department of Molecular and Translational Medicine, Brescia, Italy*

Email: giada.sebastiani@mcgill.ca

Background and aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a new positive, widely supported and not stigmatizing definition of hepatic steatosis, not requiring the exclusion of secondary causes of liver diseases, such as HIV and viral hepatitis. Liver fibrosis is a dynamic process recognized as the main predictor of liver disease progression and mortality. We aimed to investigate the effect of MASLD on liver fibrosis progression and regression in virus-related liver disease.

Method: We included consecutive people with HIV, with and without coinfection with HCV and HBV, and with at least two transient elastography examinations with controlled attenuation parameters (CAP) from three prospective cohorts in Canada, Italy, and Germany. MASLD was defined as the presence of hepatic steatosis (CAp >248 dB/m) and at least one metabolic abnormality, according to a recent multi-society Delphi consensus statement. Fibrosis progression was defined as development of significant liver fibrosis, defined as liver stiffness measurement (LSM) >8 kPa, or transition to cirrhosis, defined as LSM >13 kPa, for those with LSM >8 but <13 kPa at baseline. Fibrosis regression was defined as transition to no liver fibrosis, defined as LSM <8 kPa, or to significant liver fibrosis for those with cirrhosis at baseline. Weight gain was defined as a 5% BMI increase in two consecutive visits. A continuous-time multi-state Markov model was used to describe transition across fibrosis stages. Cox regression model was used to identify predictors for liver fibrosis progression.

Results: 1183 patients were included (median age 53 years, 77% males, median duration since HIV diagnosis 18 years, 25% HIV/HCV coinfected and 4% HIV/HBV coinfected). The baseline prevalence of MASLD, significant liver fibrosis and cirrhosis was 47%, 14% and 6% respectively. During a median follow-up period of 2.5 (1.9-3.5) years, two to six annual LSM were performed. The incidence rate of fibrosis progression and of fibrosis regression was 3.4 per 100 persons-year and 1.2 per 100 person-years, respectively. In Markov model, weight gain predicted fibrosis progression and prevented its regression (see Table). On multivariable Cox regression analysis, predictors of fibrosis progression were MASLD (adjusted hazard ratio 2.50, 95% CI 1.06-5.89; p = 0.036) and weight gain (adjusted hazard ratio 2.65, 95% CI 1.32-5.26; p = 0.006), after adjusting for male sex, age, nadir CD4 cell count, coinfection with HBV and HCV and exposure to different classes of antiretroviral regimens.

Conclusion: MASLD and weight gain are the main drivers of liver fibrosis progression in virus-related liver disease, independently of HCV and HBV coinfection and antiretroviral exposure. In the global effort for liver fibrosis screening in at-risk populations, metabolic health should be prioritized in people with HIV.

Table: Markov model describing transitions of liver fibrosis (progression or regression).

	Fibrosis progression (adjusted odds ratio, 95% CI)	**Fibrosis regression** (adjusted odds ratio, 95% CI)
Age >50 years (yes vs. no)	0.99 (0.95-1.03)	0.99 (0.95-1.02)
Males (yes vs. no)	0.87 (0.36-2.09)	**0.32 (0.14-0.75)**
Weight gain (yes vs. no)	**3.11 (1.59-6.08)**	**0.29 (0.10- 0.84)**
Years since HIV diagnosis >10 years (yes vs. no)	1.09 (0.40-2.95)	1.19 (0.43-3.33)
Nadir CD4 cell count <200 cell/uL (yes vs. no)	1.03 (0.53-2.03)	0.78 (0.35-1.74)
HBV coinfection (yes vs. no)	1.79 (0.52-6.20)	0.304 (0.04-2.51)
HCV coinfection (yes vs. no)	1.65 (0.79-3.44)	0.63 (0.29-1.39)
Current exposure to integrase strand transfer inhibitors (yes vs. no)	0.61 (0.26-1.45)	0.73 (0.34-1.58)
Current exposure to protease inhibitors (yes vs. no)	0.85 (0.35-2.06)	1.17 (0.55-2.50)
Current exposure to non-nucleoside reverse transcriptase inhibitors (yes vs. no)	0.41 (0.15-1.11)	0.99 (0.45-2.18)
Current exposure to Tenofovir alafenamide (yes vs. no)	1.11 (0.55-2.26)	0.96 (0.43-2.14)

PO2-05-YI
Steatotic liver disease and SGLT2 inhibitors: improvement of hepatic fibrosis by Fibroscan in a prospective study

Jaqueline Currà[1], Rosa Lombardi[1], Annalisa Cespiati[1], Alessandro Mantovani[2], Gabriele Maffi[1], Paolo Francione[1], Felice Cinque[1], Floriana Santomenna[1], Rosanna Villani[3], Claudio Maffeis[4], Antonio Colecchia[5], Nicola Passigato[6], Alberto Ferrarese[6], Caterina Cusumano[6], Emanuela Orsi[7], Valeria Grancini[7], Giuseppina Pisano[1], Giovanni Targher[2], Gaetano Serviddio[3], Silvia Fargion[1], Anna Ludovica Fracanzani[1]
[1]Unit of Internal Medicine and Metabolic Diseases, Fondazione Ca' Granda IRCCS., Department of Pathophysiology and Transplantation Ca' Granda IRCCS Foundation, Policlinico Hospital, University of Milan, *[2]Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy*, *[3]Centro C.U.R.E, Dept. of Medical and Surgical Sciences, University of Foggia*, *[4]Pediatric Diabetes and Metabolic Disorders Unit, Department of Surgical Sciences, Dentistry, and Pediatrics, and Gynaecology, University Hospital of Verona, Verona, Italy*, *[5]Gastroenterology Unit, Department of Medical Specialities, University Hospital of Modena, University of Modena and Reggio Emilia, Modena, Italy*, *[6]Gastroenterology Unit, Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy*, *[7]Department of Medical Science, Endocrinology and Diabetes Unit, Ca' Granda IRCCS Foundation, Policlinico Hospital, University of Milan Italy*
Email: jaqueline.curra@unimi.it

Background and aims: Steatotic liver disease (SLD) is defined by the presence of hepatic fat and when associated to metabolic alterations configures the condition of metabolic dysfunction-associated steatotic liver disease (MASLD). Among all metabolic comorbidities type 2 diabetes (T2DM) is one of the main determinants of the onset and progression of hepatic fibrosis, which is the main driver of long-term adverse outcomes. Nowadays no approved therapies are available for the treatment of hepatic steatosis, but data are accumulating on the benefits of sodium glucose cotransporter 2 inhibitors (SGLT-2i) in pharmacologic or retrospective studies. Aim: to prospectively evaluate change in hepatic disease in diabetic patients with SLD/MASLD and predisposing factors.

Method: 237 diabetic patients with MASLD (mean age 67 ± 9 years, 54% male) were enrolled and re-evaluated after 5 years. Information about diabetic control, metabolic comorbidities, medications and liver disease were collected at baseline and follow-up. Hepatic steatosis was assessed by liver ultrasonography, whereas fibrosis by liver stiffness measurement (LSM) at Fibroscan®.

Results: During follow-up, an increase in LSM values compared to baseline was registered (6.0 ± 2.8 vs 5.8 ± 2.7 kPa, $p = 0.02$), despite stability of diabetic control and prevalence of other metabolic alterations except for an increase in hypertension (81% vs 73%, $p < 0.001$). In particular, LSM worsened in 133 (56%) subjects, with 92 (39%) having a worsening of >10% from baseline and 20 (8%) of at least 1 fibrosis stage at Fibroscan from baseline. Moreover, a higher prescription of SGLT2i was seen (21% vs 6%, $p < 0.001$). Compared with those with no worsening of LSM, patients with worsening of LSM had a higher prevalence of increase in BMI during follow-up (45% vs 32%, $p = 0.06$). In multivariate analysis, after adjustment for age, sex, liver enzymes and HbA1c, the use of SGLT2-inhibitors at follow-up (adjusted-hazard ratio 0.34, 95% CI 0.13-0.91) was associated with a reduced risk of worsening of LSM by Fibroscan, even if considered>10% from baseline. However, this association was markedly attenuated after further adjusting for change in BMI over time.

Conclusion: Despite a high prevalence of fibrosis progression in SLD/MASLD subjects, we reported a potential effect of SGLT2-inhibitors in reducing the risk of worsening of liver stiffness, possibly also mediated by weight loss. Therefore, our data suggest that using this category of antidiabetic drug in SLD/MASLD patients may prevent progression of fibrosis, especially if weight control is obtained in these patients.

PO2-08
Statin treatment reduces all-cause mortality in elderly with increased risk of liver fibrosis: report from the PROSPER trial

Vivian de Jong[1 2], Stella Trompet[3], Willy Theel[4], J. Wouter Jukema[5], Diederick Grobbee[1 2], Manuel Castro Cabezas[2 4 6]
[1]University Medical Center Utrecht, Julius Center for Health Sciences and Primary Care, Utrecht, Netherlands, *[2]Julius Clinical, Science, Zeist, Netherlands*, *[3]Leiden University Medical Centre, Department of Cardiology and Department of Gerontology and Geriatrics, Leiden, Netherlands*, *[4]Franciscus Gasthuis and Vlietland, Department of Internal Medicine, Rotterdam, Netherlands*, *[5]Leiden University Medical Center, Department of Vascular medicine, Leiden, Netherlands*, *[6]Erasmus MC Medical Center, Department of Endocrinology, Rotterdam, Netherlands*
Email: v.d.dejong-5@umcutrecht.nl

Background and aims: Metabolic dysfunction-Associated Steatotic Liver Disease (MASLD), and especially liver fibrosis, are associated with an increased risk of both liver-related and cardiovascular complications. A recent meta-analysis demonstrated that statins, although under-prescribed, could be beneficial in the MASLD patient population. The cardiovascular disease (CVD) risk of MASLD in elderly subjects remains understudied.

Method: data from the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) was re-analysed. PROSPER was a double-blind RCT comparing pravastatin to placebo in an elderly population (>70 years) at increased risk of CVD. The composite end point was MACE. Single end points were: fatal/non-fatal stroke and TIA; fatal/non-fatal MI; heart failure; all-cause mortality; cancer mortality and lastly CV mortality. FIB-4 was calculated for all participants using age-adjusted cut-offs: low risk of advanced fibrosis (FIB-4 <2.0), intermediate risk (2.0 ≤ FIB-4 ≤ 2.66) and high risk (FIB-4 ≥ 2.67). Time-to-event data was analysed using the Cox proportional hazards model, for the placebo and pravastatin groups separately. The final model was adjusted for sex, smoking, BMI, diabetes and history of vascular disease.

Results: most participants were classified in the low FIB-4 group (n = 3549), followed by the intermediate group (n = 1132) and fewest in the high FIB-4 group (n = 518). The mean (± SD) age of the groups ranged from 75.0 (± 3.3) to 76.3 (± 3.5). No difference in history of diabetes was reported between groups. An increasing prevalence of a history of CVD was seen from the low (42%), to the intermediate (47%) to the high (53%) groups. Regarding medication, use of metformin and insulin did not differ between the groups. The high risk group more frequently used ACE-/Angiotensin II-inhibitors. In the placebo group, the risk of mortality increased with higher FIB-4 classification. Compared to the low FIB-4 group (reference), the intermediate group had a HR of 1.36 [CI: 1.02-1.81] and the high FIB-4 group a HR of 1.56 [1.10-2.21] for all-cause mortality. In the pravastatin group the hazard ratios did not increase with a higher FIB-4 classification, indicating a beneficial effect of pravastatin. Compared to the low FIB-4 group (reference), the intermediate group had a HR of 0.97 [0.72-1.31] and of the high FIB4-group a HR of 0.99 [0.66-1.50] for all-cause mortality. High FIB-4 classification was not statistically significantly associated with MACE or any of the other single end points in both the treatment and placebo group.

Conclusion: a higher FIB-4 classification is associated with increased all-cause mortality in elderly. Pravastatin treatment appears to abolish this risk, suggesting that treatment with statins should be considered in elderly with evidence of liver fibrosis.

PO2-09
Assessing the repeatability and reproducibility of LiverMultiScan metrics cT1 and PDFF

Cayden Beyer[1], Anneli Andersson[1], Elizabeth Shumbayawonda[1], Andrea Dennis[1], Kathleen Corey[2]
[1]*Perspectum, United Kingdom*, [2]*Massachusetts General Hospital, Boston, United States*
Email: cayden.beyer@perspectum.com

Background and aims: Multi-parametric magnetic resonance imaging (mpMRI)-derived metrics iron-corrected T1 (cT1) and proton density fat-fraction (PDFF) have proven to be excellent non-invasive biomarkers for diagnosis and monitoring of non-alcoholic steatohepatitis (NASH). They are both commonly utilized as efficacy end points in NASH trials to assess therapy-induced changes in liver health, and cT1 additionally predicts clinical outcomes. Here we assess the robustness of both metrics by investigating their test-retest variability in a NASH population.

Method: Patients were recruited at Massachusetts General Hospital. All participants underwent two LiverMultiScans within the same scanning session, five minutes between scans without exiting the scanner, (timepoints A and B, respectively) to assess the measurement variability of cT1 and PDFF. Additionally, patients histologically classified as having at-risk NASH (NAS ≥ 4 and fibrosis ≥ 2) were invited back for a third scan 2-4 weeks after their first visit (timepoint C) to test the biological/physiological variability. Bland-Altman analysis was performed, and the coefficient of variance (CoV), repeatability coefficient (RC), bias and upper/lower limits of agreement (LoA) were calculated, PDFF values are reported in relative percentages, rather than absolute.

Results: Of the patients included in this study (n = 22; age = 48 years; BMI = 36.1 kg/m^2; cT1 = 858ms; PDFF = 12.8%; 50% female, 41% diabetic), 7 had histologically confirmed at-risk NASH and were scanned 2-4 weeks following the initial scan. cT1 demonstrated high repeatability across timepoints A and B (CoV: 1.5%; RC: 32.1ms; bias: -6.2ms; 95% LoA: -36.6 to 24.2ms), and high reproducibility across timepoints A and C (CoV: 2.6%; RC: 65.2ms; bias: 6.3 ms; 95% LoA: -63.8 to 76.5ms). PDFF also demonstrated high repeatability across timepoints A and B (CoV: 1.9%; RC: 5.1%; bias: 1.1%; 95% LoA: -3.7 to 5.8%%), and high reproducibility across timepoints A and C (CoV: 6.9%; RC: 19.5%; bias: -1.1%; 95% LoA: -22.0 to 19.7%).

Conclusion: Both cT1 and PDFF are used as efficacy end points in clinical trials with meaningful changes aligning with approved end points of NASH resolution and/or fibrosis regression reported to be >80ms for cT1 and ≥30% relative change for PDFF. In this study of test-re-test variability, both metrics showed measurement variability well under the clinical meaningful change thresholds, both within session and two-weeks later with excellent repeatability and reproducibility, and proven correlations with respective histopathological features of NASH, cT1 and PDFF represent robust and reliable metrics for characterizing and assessing changes in liver tissue.

Figure:

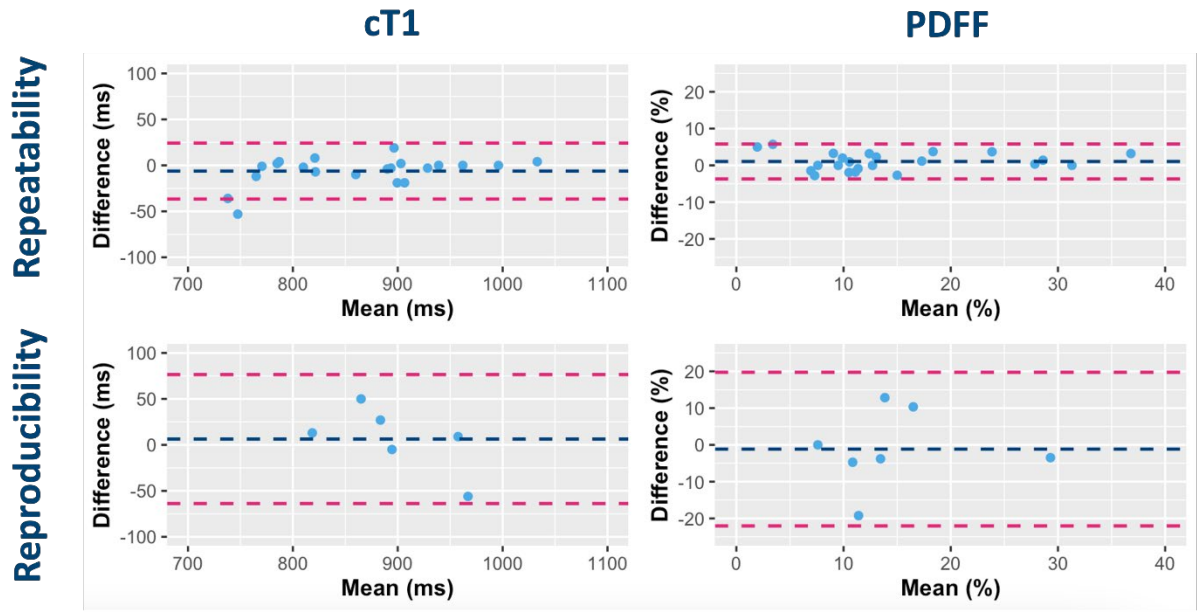


Figure 1. Bland-Altman plots showing the cT1 and PDFF differences between baseline (timepoint A) and follow-up (timepoint B for repeatability and timepoint C for reproducibility). The dashed lines represent the the upper 95% LoA (top), the mean (middle) and the lower 95% LoA (bottom).

PO2-10-YI
Hepatic steroids metabolism reflects the entity of liver damage in patients with metabolic dysfunction-associated steatohepatitis (MASH)

Eleonora Dileo[1], Chiara Rosso[1], Mirko Parasiliti-Caprino[1], Marta Guariglia[1], Gian Paolo Caviglia[1], Federico Ponzetto[1], Angelo Armandi[1], Nuria Pérez Diaz del Campo[1], Gabriele Castelnuovo[1], Maria Lorena Abate[1], Antonella Olivero[1], Giorgio Maria Saracco[1], Mauro Maccario[1], Elisabetta Bugianesi[1]
[1]*University of Turin, Medical Sciences, Turin, Italy*
Email: eleonora.dileo@unito.it

Background and aims: Circulating steroids are associated with metabolic derangements and with hepatic fibrosis in patients with metabolic dysfunction-associated steatohepatitis (MASH). The liver synthetized most of the enzymes involved in steroids metabolism. For example, hydroxysteroid dehydrogenases family includes a plethora of isoforms that are involved in different metabolic pathways such as lipid and steroid metabolism. Specifically, 11β-HSD2 is involved in the maintenance of the equilibrium between cortisone and cortisol and its inhibition is associated with an improvement of hepatic fibrogenesis, while a single nucleotide polymorphism in the gene encoding for the 17β-HSD13 isoform is protective in patients with steatohepatitis. Considering all these evidences, we hypothesized that the study of steroid metabolism in the liver may help to elucidate their pathophysiological role in the progression of MASH.

Method: In 29 biopsy-proven MASH patients (median age 55y [range 20-67], 48% male) who underwent steroidomic analysis, we performed a gene expression study by SYBR Green Real-time PCR (CFX, BioRad). We analyzed several target genes involved in the hepatic steroids metabolism (UGT2B17, UGT2B7, UGT2B4, STS, and 11βHSD2). The hepatic expression for each target was derived by the $2^{-\Delta\Delta Ct}$ method. Histology was scored according to Kleiner.

Results: Overall, 10 patients (34%) were diabetic and 6 (20.7%) were obese. Most of the patients (83%) had mild/moderate inflammation, 13 patients (45%) had moderate/severe steatosis (≥33%) and 15 subjects (51.7%) had severe fibrosis (F3/F4). Hepatic expression of SULT2A1 and UGT2B17 inversely correlated with the degree of liver steatosis ($r = -0.38$, $p = 0.043$ and $r = -0.45$, $p = 0.015$) suggesting that hepatic fat may inhibits the inactivation of active metabolites, thus promoting hepatotoxic effects. Consistently, STS enzyme, which has a sulfatase activity, was more expressed in patients with mild/moderate inflammation compared to the counterpart (3.73 *vs*. 1.63, $p = 0.012$). Interestingly, hepatic expression of 11βHSD2 was significantly higher in patients with severe hepatic fibrosis (F3/F4) compared to those with mild fibrosis (2.62 *vs*. 1.61, $p = 0.013$). Moreover, at logistic regression analysis adjusted for age, BMI and type 2 diabetes, 11βHSD2 mRNA expression in the liver was significantly associated with sever fibrosis (OR = 4, 95% CI = 1.1-14.5, $p = 0.033$).

Conclusion: Our results suggest that, in patients with MASH, the hepatic expression of such enzymes involved in steroids metabolism may impact on the progression of liver damage favoring the accumulation of active toxic metabolites.
This research has been supported by the Italian MIUR under the programme "Dipartimenti di Eccellenza 2018-2022", project code D15D18000410001 and by the University of Torino, grant number ROSC_RILO_21_01.

PO2-11-YI

Adherence to mediterranean diet reduces steatotic liver disease risk but does not affect liver fibrosis assessed by transient elastography: results from a prospective study

Gediz Dogay Us[1 2], Ozgur Koc[3], Francesco Innocenti[4], Ger Koek[1 3]
[1]Maastricht University, School of Nutrition and Translational Research in Metabolism, Maastricht, Netherlands, *[2]Pax Clinic, Department of Gastroenterology, Istanbul, Turkey*, *[3]Maastricht University Medical Center, Department of Internal Medicine, Division of gastroenterology and hepatology, Maastricht, Netherlands*, *[4]Maastricht University, Department of Methodology and Statistics, CAPHRI Care and Public Health Research Institute, Maastricht, Netherlands*
Email: dogaygediz@gmail.com

Background and aims: Diet plays a crucial role in the development, progression, and management of steatotic liver disease (SLD). Previous studies have suggested that adhering to a Mediterranean diet (MedDiet) can help prevent the condition, reduce liver fat accumulation, and improve liver health. Although the majority of the existing evidence that investigated the association between MedDiet and SLD are based on indices, current clinical guidelines require evidence of intrahepatic steatosis on imaging or histology for diagnosing SLD. Therefore, we investigated the relationship between adherence to MedDiet and liver steatosis and liver stiffness (LS) assessed by transient elastography (TE) in a population at risk of SLD.

Method: We recruited 450 adult participants for a single-centered prospective study in an outpatient clinic during a one-year period. All participants presented at least with one component of the metabolic syndrome. Physical examination, anthropometrical assessment and blood analysis were performed in a standardized clinical research visit. Individuals with excess alcohol intake were excluded. Liver steatosis by Controlled attenuation parameter (CAP), and liver stiffness were assessed using a Vibration controlled transient elastography (VCTE) device (FibroScan®; Echosens, Paris, France). SLD was defined as CAP≥ 248 db/M and fibrosis as liver stiffness measurements (LSM) ≥ 7.2 kPa in XL probe and ≥ 7.9 kPa in M probe. Adherence to MedDiet was assessed by Mediterranean diet adherence score (MEDAS), a 14-point validated scale. Univariable logistic and linear regression models were used to investigate the association of SLD and LSM with MEDAS, respectively. In multivariable models, following potential confounders were controlled: age, sex, marital status, education, income level, alcohol, smoking, obesity, high waist circumference (WC), diabetes, hypertension, dyslipidemia and metabolic syndrome.

Results: Mean MEDAS was 6.37 ± 2.15. The percentage of participants with a low, moderate, and high MEDAS were 37.6, 53.6 and 8.9%, respectively. In a crude model, one point increase in MEDAS presented a 12% decrease in the odds of having SLD (OR: 0.876, 95% CI: 0.802, 0.957). After adjusting for age, sex, marital status, education, income, obesity, high waist circumference, diabetes, hypertension, dyslipidemia and metabolic syndrome, this increased to 16% (OR: 0.840, 95% CI: 0.751, 0.939). Other significant predictors of SLD were male gender (OR: 0.560, 95% CI: 0.329, 0.953), obesity, (OR: 3.317, 95% CI: 1.956, 5.625), metabolic syndrome (OR: 2.181, 95% CI: 1.081, 4.401), and high WC (OR: 2.864, 95% CI: 1.279, 6.409). Univariable and multivariable linear regression models did not show a statistically significant and clinically relevant association between MEDAS and LSM.

Conclusion: Even a modest increase in adherence to MedDiet reduces the odds of SLD independently from obesity and all components of metabolic syndrome. Further studies demonstrating the impact of MedDiet in liver-related outcomes are warranted.

PO2-12-YI
Comparison of the prognosis of patients with metabolic dysfunction-associated steatotic liver disease and alcohol-related liver disease who underwent hepatic vein catheterization

Šimon Dostál[1], Barbora Nováková[1], Vaclav Smid[1], Jaromír Petrtýl[1], Radan Brůha[1]
[1]General university hospital in Prague, 4th internal medicine clinic, Praha 2
Email: dostal.sim@seznam.cz

Background and aims: Alcohol-related steatotic liver disease (MetALD) and metabolic dysfunction-associated steatotic liver disease (MASLD) represent the most common liver diseases in the Czech population. There are not many studies directly comparing the prognosis of these two categories of liver disease, however the prognosis of patients with alcoholic etiology seems to be worse. We aimed to compare the survival of patients with MetALD and MASLD in relation to the severity of portal hypertension.

Method: Among patients referred between 2003 and 2021 to our institution for hepatic vein catheterization, mostly also with transjugular liver biopsy, we identified 206 patients with MetALD and 49 patients with MASLD in the stage of advanced liver disease. A retrospective analysis of the survival of these patients in relation to the severity of portal hypertension and other variables was performed. Both etiologic groups were further split into subgroups with low and high hepatic vein pressure gradient (HVPG <12 and ≥12 mmHg respectively).

Results: Overall, the prognosis of patients with MASLD is significantly better compared to patients with MetALD (p <0.01). Patients with MASLD were catheterized at an older age (median 61 vs 57 years in MetALD), yet we observed both longer survival from catheterization (median 8 vs 5.6 years in MetALD) and longer overall life expectancy of MASLD patients (median 69 vs 62 years in MetALD). In subgroup analysis according to hepatic vein pressure gradient (HVPG) we observed a trend towards shorter survival with higher HVPG, however this difference has reached statistical significancy only in MASLD patients (p = 0.013). When compared to respective gender and age matched general populations both MetALD subgroups and MASLD subgroup with HVPG ≥12 mmHg showed significantly worse survival (p <0.01), whereas MASLD subgroup with HVPG <12 mmHg had comparable survival with its gender and age matched general population despite comprising patients with histologically proved advanced liver disease. Interestingly MASLD subgroup with HVPG ≥12 mmHg although being significantly older at catheterization (median age 64 vs 57 years in MetALD ≥12 mmHg) has showed similar survival from catheterization with MetALD group with HVPG <12mmHg.

Conclusion: Overall, the prognosis of patients with MASLD is significantly better compared to patients with MetALD. The correlation with severity of portal hypertension was not as strong as we expected and is mostly seen in the MASLD group.
Supported by: AZV-MHCR-NU23-01-00288, MH CZ-DRO-VFN64165 and Cooperatio program (research area DIAG) given by the Charles University

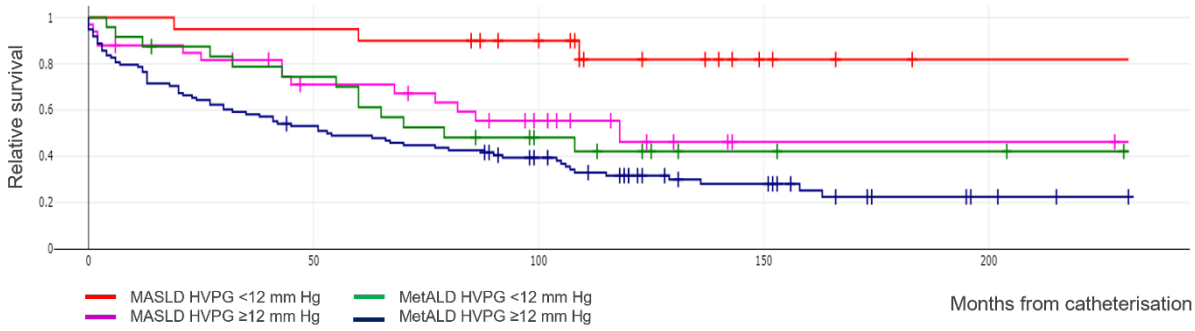


Figure: Comparison of the prognosis of patients with metabolic dysfunction-associated steatotic liver disease and alcohol-related steatotic liver disease. Kaplan-Meier plot.

PO2-13-YI
Progression to cirrhosis and all-cause mortality is increased In postmenopausal women with MASLD independently of fibrosis stage at baseline

Aleksandra Dunin-Borkowska[1 1], Chioma Izzi-Engbeaya[1], Pei Eng[1], Roberta Forlano[1], Benjamin H. Mullish[1], Giordano Sigon[1], Vicky Lin[1], Tricia Tan[1], Michael Yee[2], Pinelopi Manousou[1], Waljit Dhillo[1]
[1]*Imperial College London, United Kingdom*, [2]*Imperial College Healthcare NHS Trust, United Kingdom*
Email: ad6618@ic.ac.uk

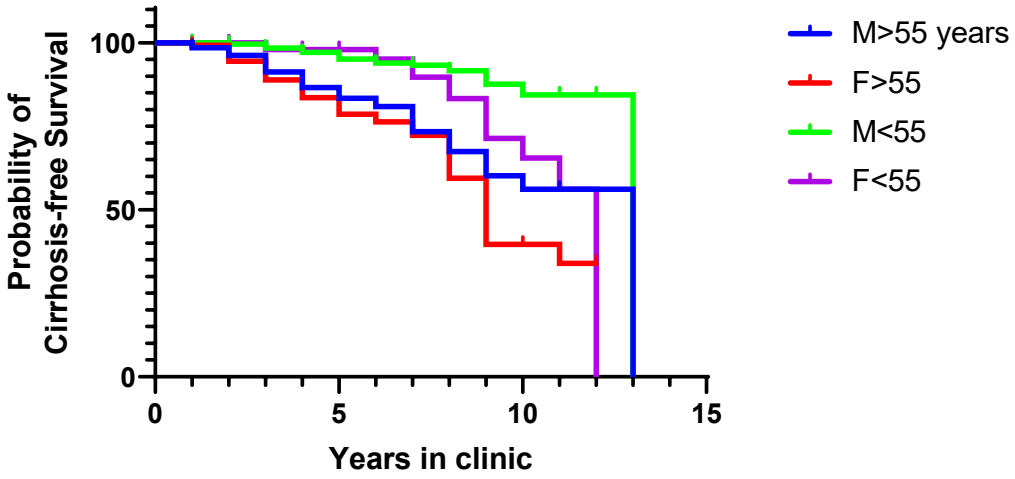
Background and aims: Metabolically Associated Steatotic liver disease (MASLD) has become a silent worldwide pandemic, affecting up to 30% of adults. Progression to advanced fibrosis and cirrhosis are strongly associated with adverse outcomes, including significantly increased rates of liver-related and non-liver related (cardiovascular and extra-hepatic cancers) mortality. Worryingly, recent epidemiological data indicate an increasing trend in MASLD-related mortality rates and more frequent liver decompensation events in postmenopausal women with MASLD. However, the causes of these outcomes are incompletely understood.

Method: We included patients reviewed between January 2009 and December 2020 at the MASLD clinic, Imperial College Healthcare NHS Trust. Data were collected prospectively in clinic and retrospectively from the electronic health records until December 2022. Female patients were classified as postmenopausal if aged ≥55 years. Cirrhosis was diagnosed based on biopsy results or a combination of clinical and radiological findings. Statistical significance was set at $p < 0.05$. Multivariate logistic regression was utilised to identify variables independently associated with the diagnosis of cirrhosis and mortality.

Results: Of 739 patients included in this analysis, 286 were women (39%). Median (IQR) age of participants was 52 (42-60) years, with a median follow-up of 6 (3-8) years. At first clinic visit, 73% of postmenopausal women (n = 153) had a FIB-4 score of over 1.3, compared to 85% of men aged ≥55 years ($p <0.0001$). There were similar proportions of baseline fibrosis stage ≥2 in postmenopausal women compared to age matched men (82% vs 78% respectively, $p = 0.55$), but significantly more compared to pre-menopausal women (82% vs 50% respectively, $p < 0.001$). Initial prevalence of co-morbidities was similar between postmenopausal women and age-matched men, including prevalence of obesity ($p = 0.63$), type 2 diabetes ($p = 0.15$) and cardiovascular disease ($p = 0.21$). However, at last follow-up, postmenopausal women had significantly higher rates of diagnosis of cirrhosis compared to age-matched men (36% vs 25% respectively, $p < 0.05$). Furthermore, all-cause mortality was higher amongst postmenopausal women than age-matched men (14% vs 9% respectively, $p < 0.0001$). There were insufficient data on reproductive hormone use for this variable to be included in analyses.

Conclusion: Compared to age-matched men, postmenopausal women with MASLD were more likely to progress to cirrhosis despite similar fibrosis stage at baseline and both groups receiving similar treatments following diagnosis. This finding was independent of co-morbidities at baseline between the two groups. Therefore, tailored diagnostic and management strategies may be required to improve outcomes in postmenopausal women with MASLD.

Figure: Probability of Cirrhosis-free Survival ($p < 0.0001$ for the difference between all 4 curves)



PO2-16
The effect of baseline hepatic steatosis on fibrosis changes after SVR in CHC-Genotype 4 patients treated with INF, a 10-year follow-up

Mohammed Elbasiony[1 2], Reham Soliman[2 3], Nabil Mikhail[2 4], Gamal Shiha[1 2 2]
[1]Hepatology and Gastroenterology unit, Internal Medicine Department, Faculty of Medicine, Mansoura University, Egypt, *[2]Egyptian Liver Research Institute and Hospital (ELRIAH), Sherbin, ElMansoura, Egypt*, *[3]Tropical Medicine Department, Faculty of Medicine, Port Said University, Port Said, Egypt*, *[4]Biostatistics and Cancer Epidemiology Department, South Egypt Cancer Institute, Assiut University,*
Email: elbasiony.m@gmail.com

Background and aims: Steatosis leads to disease progression in chronic hepatitis C (CHC) patients, however, its effect on fibrosis after INF-induced SVR was not studied. In addition, there is no long-period follow-up studies for CHC-Genotype 4 patients. Our aim was to Study the effect of baseline steatosis on fibrosis changes in CHC-Genotype 4 patients treated with interferon (INF), in a 10-year follow-up study

Method: A retrospective single-center study was conducted at the Association of Liver Patients Care, El Mansoura, Egypt, in patients with CHC-Genotype 4 infection treated with INF (from Jan 2007 to November 2011). The changes of fibrosis stages were studied. Outcomes of all-cause mortality, Hepatocellular carcinoma, and decompensation of cirrhosis have been also evaluated. Analysis of the factors affecting outcomes was done with special focus on steatosis.

Results: A total of 1292 patients were included, and data of 595 was analyzed. SVR rate of 64.8%was found. The median time of patient follow-up was 120 months (IQR 115.0-126.0). 140 patients (26%) showed improvement of fibrosis. 49 patients (9.1%) of them underwent reversal of fibrosis, among them 34 patients (69.4%) had SVR (p = 0.001), 37 patients (75.5%) had no baseline steatosis (p = 0.009), and 26 patients (76.5%) within SVR group had no basal steatosis (p = 0.066). In addition, 91 patients (16.9%) showed regression of fibrosis, among them 59 patients (64.8 %) had SVR (p = 0.001), 79 patients (86.8%) had no baseline steatosis (p = 0.009), and 52 patients (88.1%) within SVR group had no baseline steatosis (p = 0.66). 308 patients (57.2%) showed stationary course of fibrosis, among them 179 patients (58.1%) had SVR (p = 0.001), 254 patients (82.5%) had no baseline steatosis (p = 0.009), and 152 patients (84.9%) in SVR group had no steatosis (p = 0.066). On the other hand, 90 patients (16.7%) showed progression of fibrosis, 36 (40%) of them didn't achieve SVR (p = 0.001), 28 patients (31.1%) had baseline steatosis (p = 0.009), and 11 (30.6%) of those who achieved SVR had baseline steatosis (p = 0.066).DM increased all-cause mortality (HR = 2.5%), HCC (HR = 2.4%), and decompensated cirrhosis (HR = 3.9%). 57 patients died during the study; 48 liver-related and 9 as non-liver-related. 40 cases of hepatocellular carcinoma and 41 cases of decompensated cirrhosis were developed. Viral clearance was associated with a decrease in all-cause mortality (HR 0.194, 95% CI 0.058-0.646; p = 0.008), hepatocellular carcinoma (HR 0.081, 95% CI 0.011-0.614; p = 0.015), and decompensated cirrhosis (HR 0.094, 95% CI 0.013-0.706; p = 0.021).

Conclusion: Absence of baseline steatosis favors fibrosis outcomes in CHC-Genotype 4 patients treated with INF. Moreover presence of steatosis could explain fibrosis progression in some of these patients. In addition, INF-induced SVR improve overall mortality, HCC, and hepatic decompensation.

Figure:

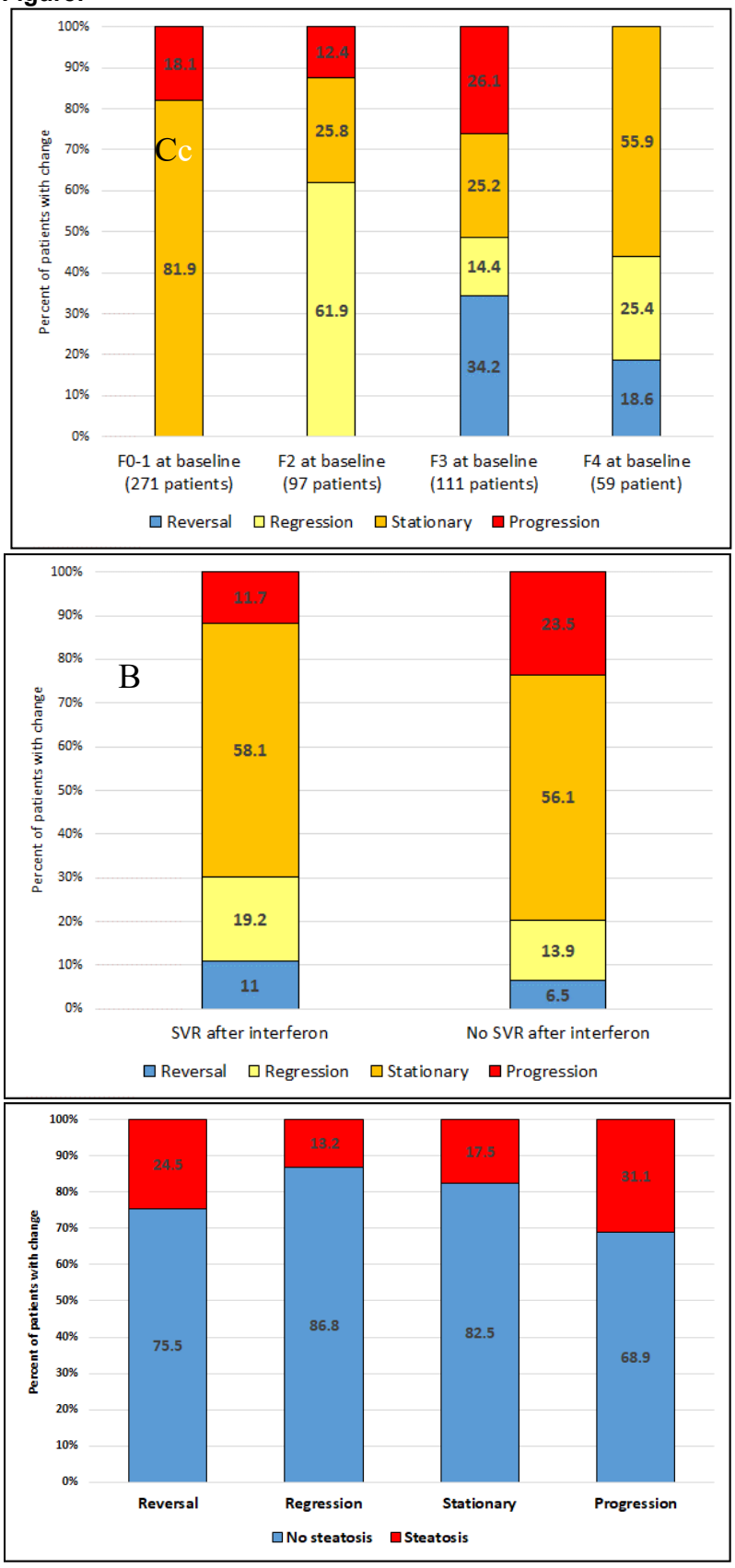


Figure : Changes in hepatic fibrosis in 538 patients attended follow-up visit, according to: (a) baseline fibrosis; (b) SVR after interferon-based treatment course; and (c) steatosis

PO2-17-YI
Assessing the diagnostic accuracy of the fibroScan-AST (FAST) score for high-risk metabolic dysfunction-associated steatohepatitis : a diagnostic accuracy meta-analysis

Muhammed Elfaituri[1], Ala Khaled[1], Ahmed BenGhatnsh[1], Hazem Faraj[1], Ahmed Msherghi[1]
[1]*University of Tripoli, Faculty of Medicine, Tripoli, Libya*
Email: dr.muhammed.khaled.elfaituri@gmail.com

Background and aims: Metabolic dysfunction-associated steatohepatitis (MASH), a progressive form of metabolic dysfunction-associated steatotic liver disease (MASLD), has the potential to advance to cirrhosis and hepatocellular carcinoma. Early and precise detection of high-risk MASH patients is vital for effective intervention and management. The FibroScan-AST (FAST) score, which incorporates liver stiffness measurement and aspartate aminotransferase levels, has been suggested as a potential diagnostic tool. However, a comprehensive assessment of its diagnostic accuracy is still required. This study aims to rigorously evaluate the diagnostic accuracy of the FAST score in identifying high-risk MASH patients.

Method: An extensive search of electronic databases, including PubMed, EMBASE, and the Cochrane Library, was conducted for studies published until December 2022 that utilized the FAST score for MASH detection. Studies providing sufficient data to compute diagnostic odds ratios were included. We set a cut-off FAST score of ≥0.67 to rule in high-risk MASH patients. The analyses were performed using R software (version 4.0.3) with the mada package, facilitating the pooling of sensitivity, specificity, false-positive rate estimates, diagnostic odds ratio, positive Likelihood Ratio (LR), and negative Likelihood Ratio (LR), all of which are presented with a 95% Confidence Interval (CI). The I^2 statistic was employed to evaluate heterogeneity among the studies.

Results: Our search yielded a total of 11 studies encompassing 4459 patients, of which 1513 had confirmed MASH. The FAST score, using a cutoff of ≥0.67, demonstrated a pooled sensitivity of 44.1% (95% CI: 34.9-53.8%, I^2 = 83.4%) for identifying high-risk MASH, which indicates moderate capability in correctly identifying patients with MASH. The pooled specificity was 88.9% (95% CI: 81.2-93.7%, I^2 = 92.7%), suggesting a high ability to correctly identify patients without MASH. The false-positive rate was 11.1% (95% CI: 6.3-18.8%). The diagnostic odds ratio was 6.33 (95% CI: 3.05-13.14), indicating a moderate association between the FAST score and the presence of MASH. The positive LR was 3.97 (95% CI: 2.20-7.18), and the negative LR was 0.63 (95% CI: 0.52-0.76), revealing that the FAST score is relatively more informative in ruling in rather than ruling out the disease.

Conclusion: This study offers a comprehensive evaluation of the diagnostic accuracy of the FAST score for detecting high-risk MASH patients. The moderate sensitivity and high specificity suggest that the FAST score may be more effective as a rule-in test than a screening tool. Furthermore, the significant heterogeneity among the studies indicates that additional research is needed to identify factors contributing to the variability and to optimize the use of the FAST score in clinical practice. This information can be instrumental in refining early detection and management strategies for MASH.

PO2-18
The hepatokine leukocyte cell-derived chemotaxin-2 is elevated in people with impaired glycaemic control and augmented by acute exercise

Buket Engin[1 2], Scott Willis[1 2], Sundus Malaikah[1 2 3], Jack Sargeant[2 4], David Stensel[1 2 5 6], Charlotte Jelleyman[7], Gael Ennequin[8], Guruprasad Aithal[9 10], Thomas Yates[2 4], James King[1 2]
[1]National Centre for Sport and Exercise Medicine, School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, United Kingdom, *[2]NIHR Leicester Biomedical Research Centre, University Hospitals of Leicester NHS Trust and University of Leicester, Leicester, United Kingdom*, *[3]Clinical Nutrition Department, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia*, *[4]Diabetes Research Centre, College of Life Sciences, University of Leicester, Leicester, United Kingdom*, *[5]Faculty of Sport Sciences, Waseda University, Tokorozawa, Japan*, *[6]Department of Sports Science and Physical Education, The Chinese University of Hong Kong, Hong Kong, China*, *[7]Human Potential Centre, School of Sport and Recreation, Auckland University of Technology, Auckland, New Zealand*, *[8]Laboratory of the Metabolic Adaptations to Exercise under Physiological and Pathological Conditions (AME2P), Clermont Auvergne University, CRNH Auvergne, Clermont-Ferrand, France*, *[9]Nottingham Digestive Diseases Centre, School of Medicine, University of Nottingham, Nottingham, United Kingdom*, *[10]NIHR Nottingham Biomedical Research Centre, Nottingham University Hospitals NHS Trust and the University of Nottingham, Nottingham, United Kingdom*
Email: b.engin@lboro.ac.uk

Background and aims: Leukocyte cell-derived chemotaxin-2 (LECT2) is a hepatokine that has been shown to promote insulin resistance (skeletal muscle and adipose tissue) and hepatic fibrogenesis. Whilst circulating levels of LECT2 are associated with obesity-related outcomes, the association with glycaemic control is less clear. In rodents, acute exercise suppresses circulating levels of LECT2; however, data in humans are lacking. This human study compared circulating levels of LECT2 across populations with varied glycaemic control, and explored whether acute exercise impacts circulating LECT2 concentration.

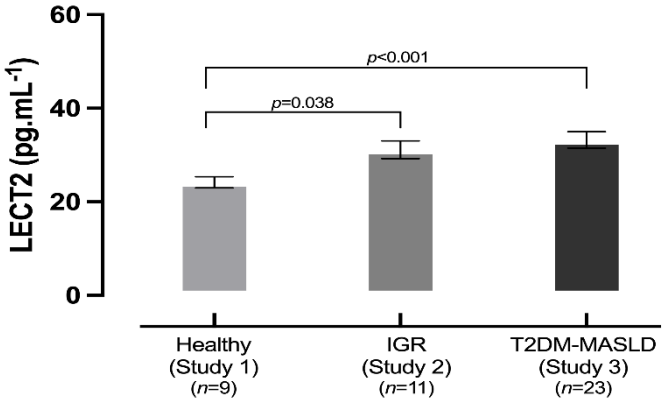
Method: Data were pooled (n = 43) from three experimental studies in either healthy individuals (18-40 y; body mass index [BMI] ≥18.5 and <30.0 kg.m^{-2}), individuals with impaired glycaemic regulation (IGR) (50-74 y; BMI ≥27.5 kg.m^{-2}), and individuals with type 2 diabetes and metabolic dysfunction-associated steatotic liver disease (T2DM-MASLD) (30-75 y; BMI ≥27.0 and ≤45.0 kg.m^{-2}). In Part A, generalised linear models assessed differences in circulating LECT2 concentrations between the three cohorts. Part B (n = 20) involved two acute experimental trials, treadmill exercise (30 min at 65% of peak oxygen uptake [VO_{2peak}]) vs control (resting), in the healthy and IGR groups. Circulating LECT2 was measured before exercise and at 0, 1, 2 and 3h post-exercise. Generalised estimating equations (GEE) were used to assess differences in LECT2 responses to exercise and control trials between study groups.

Results: In Part A, circulating LECT2 concentrations were 28.7% and 37.3% higher in the groups with IGR and T2DM-MASLD compared to healthy individuals (both p≤0.038). Univariable regression analyses showed age (p = 0.003) and BMI (p = 0.001) were positively associated, and VO_{2peak} (p = 0.004) inversely associated, with circulating LECT2, while multivariable regression analyses identified BMI as the main predictor of circulating LECT2 (p = 0.008). In Part B, GEE analysis showed that average circulating LECT2 concentrations were 6.3% higher across the exercise trial compared with control (p < 0.001); with responses being similar between groups (p = 0.079). When groups were combined, plasma LECT2 concentrations were elevated between 1-3h after exercise compared to control (all p≤0.009).

Conclusion: These findings demonstrate that circulating levels of LECT2 are elevated in people with dysglycaemia and that BMI is a leading predictor of circulating LECT2 concentrations. Furthermore, in contrast to previous rodent work, acute exercise augments rather than suppresses circulating LECT2 in humans. Future research is needed to further investigate the impact and health implications of both acute and chronic adaptations of LECT2 physiology to exercise training in humans.

Figure: a) fasting LECT2 concentrations stratified by study. b) circulating LECT2 responses during control and exercise trials in both study groups and (c) circulating LECT2 responses during control and exercise trials in the combined study population. Meals: black circles, exercise: black boxes. Data are presented as estimated marginal means ± SEM. [a]Exercise group significantly different from control group.

a)



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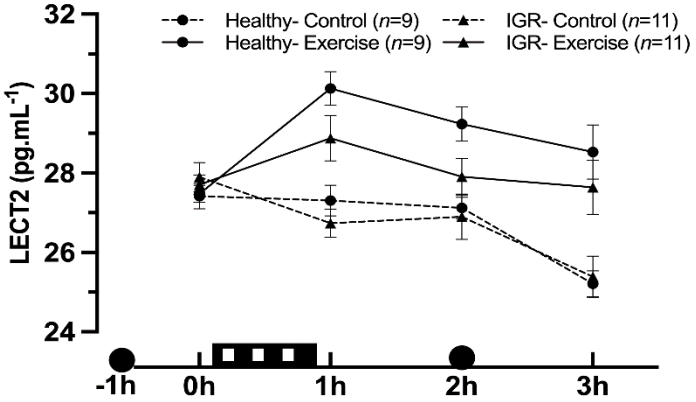
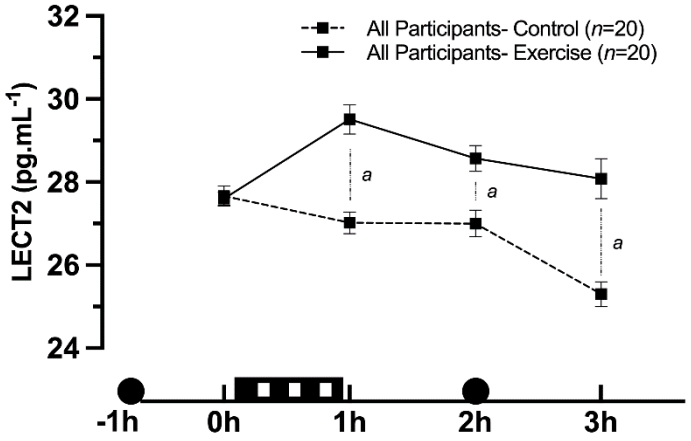


c)



PO2-19
Comparison between transient elastography and shear wave elastography for assessment of liver fibrosis in Egyptian patients with NAFLD: a single center experience

Ahmed Fayoumi[1]
[1]*National Liver Institute, Hepatology and Gastroenterology department, Shebin El-Kom, Egypt., Egypt*
Email: antarf2010@gmail.com

Background and aims: Non-alcoholic fatty liver disease (NAFLD) has become the most common form of chronic liver disease worldwide. It is associated with significant complications such as cirrhosis, hepatocellular carcinoma (HCC) and overall mortality. Transient elastography (TE) and point shear wave elastography (pSWE) are non-invasive methods to diagnose fibrosis stage in patients with chronic liver disease. We aimed to compare the accuracy of the two methods to diagnose fibrosis stage in non-alcoholic fatty liver disease (NAFLD).

Method: We enrolled 250 NAFLD patients who underwent clinical evaluation, laboratory characteristics, B-mode ultrasound and liver stiffness measurements by both point shear wave elastography and TE on the same day.

Results: The mean age of studied patients was 41.5 ± 10.7 years and male represented 56.0%. Kappa Agreement between (NAFLD TE and SWE) for F0 was (97.4%-89.4%) F1 (80.3%-85.5%) F2 (86%-91.5%) F3 (80%-92.3%) F4 (50%-100%) respectively with p value <0.0001. The AUROC of TE for diagnosis of fibrosis stage F1, ≥F2, ≥F3, and F4 were 0.75, 0.87, 0.90, and 0.91, respectively. The corresponding AUROC of pSWE was 0.73, 0.82, 0.91, and 1.000, respectively. No statistically significant differences were found between TE and pSWE for diagnosis fibrosis stage ≥F1, ≥F2 ≥F3, and F4.

Conclusion: Both Transient elastography (TE) and point shear wave elastography (pSWE) are the the same in the diagnosis fibrosis in NAFLD patients

PO3-01
PNPLA3 is associated to FIB-4 but not to (cardiovascular) mortality in an elderly population: report from the PROSPER trial

Vivian de Jong[1 2], Stella Trompet[3], Willy Theel[4], J. Wouter Jukema[5], Diederick Grobbee[1 2], Manuel Castro Cabezas[2 4 6]
[1]University Medical Center Utrecht, Julius Center for Health Sciences and Primary Care, Utrecht, Netherlands, *[2]Julius Clinical, Science, Zeist, Netherlands*, *[3]Leiden University Medical Centre, Department of Cardiology and Department of Gerontology and Geriatrics, Leiden, Netherlands*, *[4]Franciscus Gasthuis and Vlietland, Department of Internal Medicine, Rotterdam, Netherlands*, *[5]Leiden University Medical Center, Department of Vascular medicine, Leiden, Netherlands*, *[6]Erasmus MC Medical Center, Department of Endocrinology, Rotterdam, Netherlands*
Email: v.d.dejong-5@umcutrecht.nl

Background and aims: disease progression in Metabolic Dysfunction-associated Steatotic Liver Disease (MASLD) is not only associated with liver-related complications, but also with cardiovascular disease (CVD). Intra-individual variation in MASLD progression may be partly due to genetic factors. Several single nucleotide polymorphisms (SNPs) associated with MASLD, MASH and/or fibrosis have been identified in genome-wide association studies (GWAS). CVD risks and genetics are not yet well-described in elderly populations.

Method: data from the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) was re-analysed. PROSPER was a double-blind RCT comparing pravastatin to placebo in an elderly population (>70 years) at increased risk of CVD. The composite end point was MACE. A GWAS was carried out for 5244 participants of Caucasian descent. Thirty SNPs associated with MASLD/MASH/fibrosis in literature were extracted from the available GWAS data. FIB-4 was calculated for all participants using age-adjusted cut-offs: low risk of advanced fibrosis (FIB-4 <2.0), intermediate risk (2.0 ≤ FIB-4 ≤ 2.66) and high risk (FIB-4 ≥ 2.67). The association between minor allele frequencies (MAFs) and FIB-4 categories was tested with Chi-square. A Cox proportional hazards model was fitted for end points in SNP carriers vs. non-carriers (reference) in the treatment and placebo group.

Results: most participants were classified in the low FIB-4 group (n = 3549), followed by the intermediate group (n = 1132) and lastly the high FIB-4 group (n = 518). The mean (± SD) age of the groups ranged from 75.0 (± 3.3) to 76.3 (± 3.5). We replicated previous reported associations between 4 SNPs. The MAFs of SLC39A8, TRIB1, SAMM50 and PNPLA3 were significantly associated with risk classification by FIB-4. The strongest association was observed for PNPLA3 (p = 0.001). No association was found between FIB-4 classification and other well-known SNPs: MBOAT7, HSD17B13 and GCKR. No differences between the composite end point, or single end points, were observed for carriers vs. non-carriers of PNPLA3 and TRIB1 in either placebo or treatment group. The risk for fatal/nonfatal MI was increased for carriers of SLC39A8 in the placebo group, HR = 1.40 [CI: 1.07-1.83]. This risk was reduced in those randomized to statins, HR = 1.13 [0.82-1.55]. For carriers of SAMM50 no association with end points was found in the placebo group, however in the treatment group an increased HR = 1.47 [1.01-2.14] was found for fatal/non-fatal stroke/TIA and a decreased HR = 0.58 [0.35-0.97] for heart failure.

Conclusion: in this elderly population we confirm the association between high FIB-4, as indicator for the presence of liver fibrosis, PNPLA3 and several other SNPs. PNPLA3 in itself is not associated with increased risk for all-cause mortality or MACE. The increased risk on fatal/nonfatal MI for SLC39A8, was mitigated by statin use.

PO3-02-YI
Steatotic liver disease and risk of hepatocellular carcinoma in hepatitis B and C infected individuals: a systematic review and meta-analysis

Muhammed Elfaituri[1], Ahmed Msherghi[1], Ahmed BenGhatnsh[1], Ala Khaled[1], Ahmed Elfaituri[1], Hazem Faraj[1]
[1]*University of Tripoli, Faculty of Medicine, Tripoli, Libya*
Email: dr.muhammed.khaled.elfaituri@gmail.com

Background and aims: With the global prevalence of steatotic liver disease and viral hepatitis (Hepatitis B or Hepatitis C) increasing, understanding their interconnected implications for hepatocellular carcinoma (HCC) risk becomes paramount. However, the potential association between steatotic liver disease and HCC risk in individuals infected with HBV or HCV remains unexplored, necessitating further investigation. This study aims to clarify the relationship between steatotic liver disease and the propensity for HCC in patients burdened with either HBV or HCV infection.

Method: A rigorous systematic review of pertinent studies, available on PubMed, Embase, and the Cochrane Library up to December 2022, was conducted. Studies reporting individuals with either HBV or HCV infection investigated the association between steatotic liver disease and HCC were included. The meta-analysis used multivariate Hazard Ratios (HR) as the primary effect size measure, with the aid of R 4.0.3 and its corresponding packages, metafor and meta.

Results: Our analysis integrated thirteen studies. Interestingly, steatotic liver disease did not exhibit a significant association with elevated HCC risk in individuals infected with either HBV or HCV (Multivariate HR = 1.23, 95% CI 0.88-2.73, p = 0.22, I^2 = 88%), nor in those with HBV alone (Multivariate HR = 0.83, 95% CI 0.48-1.43, p = 0.51, I^2 = 86%). In contrast, a marked increase in HCC risk was observed in individuals concurrently afflicted with steatotic liver disease and HCV infection (Multivariate HR = 2.16, 95% CI 1.66-2.82, p < 0.01, I^2 = 0%), denoting a more than twofold risk compared to those without fatty liver.

Conclusion: This comprehensive analysis reveals a significant link between steatotic liver disease and an amplified HCC risk in individuals infected with HCV. However, this correlation was not apparent for HBV infection or when HBV and HCV infections were collectively analyzed. These findings highlight the urgent need for early detection and efficient management of steatotic liver disease in viral hepatitis patients to curb HCC risk. They also underscore the requirement for additional large-scale prospective studies to confirm these results and to investigate the potential mechanisms by which steatotic liver could increase HCC risk in this population.

PO3-04-YI
The natural history of metabolic dysfunction-associated steatotic liver disease with advanced fibrosis

Zouhir Gadi[1 1 2], Luisa Vonghia[1 2], Lotte Schoenmakers[1], Jolien Derdeyn[1], Thomas Vanwolleghem[1 2], Toon Steinhauser[1], Wilhelmus Kwanten[1 2], Sven Francque[1 2]
[1]Antwerp University Hospital, Department of Gastroenterology and Hepatology, Edegem, Belgium, *[2]University of Antwerp-Faculty of Medicine, Laboratory of Experimental Medicine and Paediatrics (LEMP), Antwerpen, Belgium*
Email: Zouhir.Gadi@student.uantwerpen.be

Background and aim: Data on the natural history of advanced chronic liver disease (ACLD) in metabolic dysfunction-associated steatotic liver disease (MASLD) are scarce. Therefore, we aim to describe the natural history of MASLD patients with advanced fibrosis.

Methods: We retrospectively collected patients with biopsy-proven MASLD between March 2006 and February 2022 and an available clinical follow-up time of at least 12 months after the histological confirmation of advanced fibrosis (F3-F4). Hepatic decompensation was defined as variceal bleed (VB), overt hepatic encephalopathy (HE) or ascites (As). Furthermore, the following clinically relevant events were collected: development of hepatocellular carcinoma (HCC), liver transplantation, liver-related death and death.

Results: We identified 122 patients (62 F3; 60 F4), of which 6 presented with decompensation at baseline. The remaining 116 patient had a median follow time of 5 (1-19) years. In the whole cohort 47 first decompensation events were registered in 31/122 (25%) patients distributed as As/HE/VB = 22/12/13 (resp. 18/10/11%), with 11 patients experiencing more than 1 events. Excluding patients with decompensation at baseline, decompensation was seen in 25/116 (22%) patients distributed as 19/11/8 (16/9/7%), with ≥1 event in 8 patients. Focussing on patients with stable non-decompensating disease in the first 12m of follow-up, thus excluding 3 patients, 32 events were noted in 22/113 (19%) with As/HE/VB = 17/8/7 (15/7/6%), with ≥1 event in 7 patients. In the overall 122 patients, decompensated patients, when compared to those without decompensation, had significantly higher (*i.e.* at time of diagnosis) age, spleen size, fibrosis (liver histology), potassium, bilirubin, aPPT, INR, Child Pugh Score (CPS), Na-MELD, predicted (HVPG-3P and HVPG-5P model (*Reiniš, J Hep 2023*)) and effectively measured HVPG. The platelet count, ALT, cholinesterase, serum sodium and steatosis (histology) were significantly decreased. When excluding patients with decompensation at baseline or within the first 12m follow-up, the following baseline parameters were significantly different In those who developed decompensation: age, spleen size, platelet count, potassium, INR, CPS, Na-MELD, predicted (HVPG-3P and HVPG-5P model) and HVPG. Interestingly, 5/29 (17.2%) patients with decompensation had F3 fibrosis and 11/28 (39%) had a HVPG <10 mmHg. HCC (4 cases) with a mean follow-up time of 211 (29-473) weeks and 23 deaths (8/23 liver-related) with a mean follow-up time 228 (55-733) weeks were noted. Of these cases 1/4 and 14/23 experienced prior decompensation.

Conclusion: In this large cohort one-fifth developed a first decompensating event with median follow-up of 5 years. Ascites was the most frequent decompensation event. Age, liver function and portal hypertension related parameters at baseline were associated with decompensation.

PO3-08-YI
Culturally adapting the mediterranean diet for the hispanic and latino population in the weight intervention in liver disease (WILD) clinical pathway

Sheena Gillani[1], Nancy S Reau[2], Sujit Janardhan[2], Colleen Folkers[2], Sarah Repking[2], Laura Ritchie[2], Sheila Eswaran[2]
[1]RUSH University Medical Center, Chicago, United States, *[2]RUSH University Medical Center, Division of Digestive Diseases and Nutrition, Chicago, United States*
Email: sheena_h_gillani@rush.edu

Background and aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) carries a prevalence of 35% in the United States (US) and disproportionately affects the Hispanic and Latino populations. Lifestyle interventions are the gold standard for treatment, with a weight loss goal of 5-10% needed to achieve liver disease regression. Although the Mediterranean diet is the most evidence-based regimen, its recommendations are geared towards Western diets. As ethnic diversity increases in the US, so does the need for culturally relevant dietary recommendations. The Weight Intervention in Liver Disease (WILD) clinical pathway is a clinically proven weight loss intervention for MASLD patients located at a tertiary care center in Chicago. The purpose of this study is to analyze the WILD clinic population to determine whether the addition of culturally relevant Mediterranean diet menus would be beneficial.

Method: From October 2018 to November 2022, 72 patients with MASLD were enrolled into the WILD clinic and had follow-up with 2+ visits or >10% weight loss over 6 months. De-identified demographic data and body mass index (BMI) were exported from the electronic health record into an excel spreadsheet. The primary outcomes measured include the final percentage of weight loss and percentage of patients with >10% weight loss. Student's t-Test was used to compare outcomes between demographics groups. Simultaneously, culturally adapted Mediterranean diet menus for the East Asian, South Asian, and Hispanic and Latino populations were developed using the USDA Mediterranean Diet guidelines.

Results: Of the 72 patients, a majority (75%) were female, and the average age was 53 years old (26-76 years old). For demographics, 39% were Hispanic and Latino, with 31% non-Hispanic white, 17% African American, 10% Asian, and 4% other. The average BMI was 37 kg/m^2 (23.99-58.51 kg/m^2) and the average number of visits was 7 over a 13-month period. At the end of the study, the average final weight loss percentage was 4.8%. Although not statically significant, non-Hispanic whites had a greater final percentage of weight loss compared to Hispanic and Latinos (5.4%, 3.4%, $p = 0.33$). Additionally, 22% of non-Hispanic whites had >10% weight loss compared to 14% of Hispanic and Latinos. Given this disparity, the Hispanic and Latino menu (Figure 1) will be distributed over the course of September 2023 to March 2024 to WILD patients and non-WILD patients to see if culturally relevant dietary recommendations improve weight loss compared to standard of care.

Conclusion: The Mediterranean diet is recommended for the treatment of MASLD; however, adherence can be limited due to cultural differences. We propose adaptations to the Mediterranean diet keeping in mind culture preferences to improve long term adherence, achieve a greater weight loss goal, and prevent the progression of MASLD.

Figure:

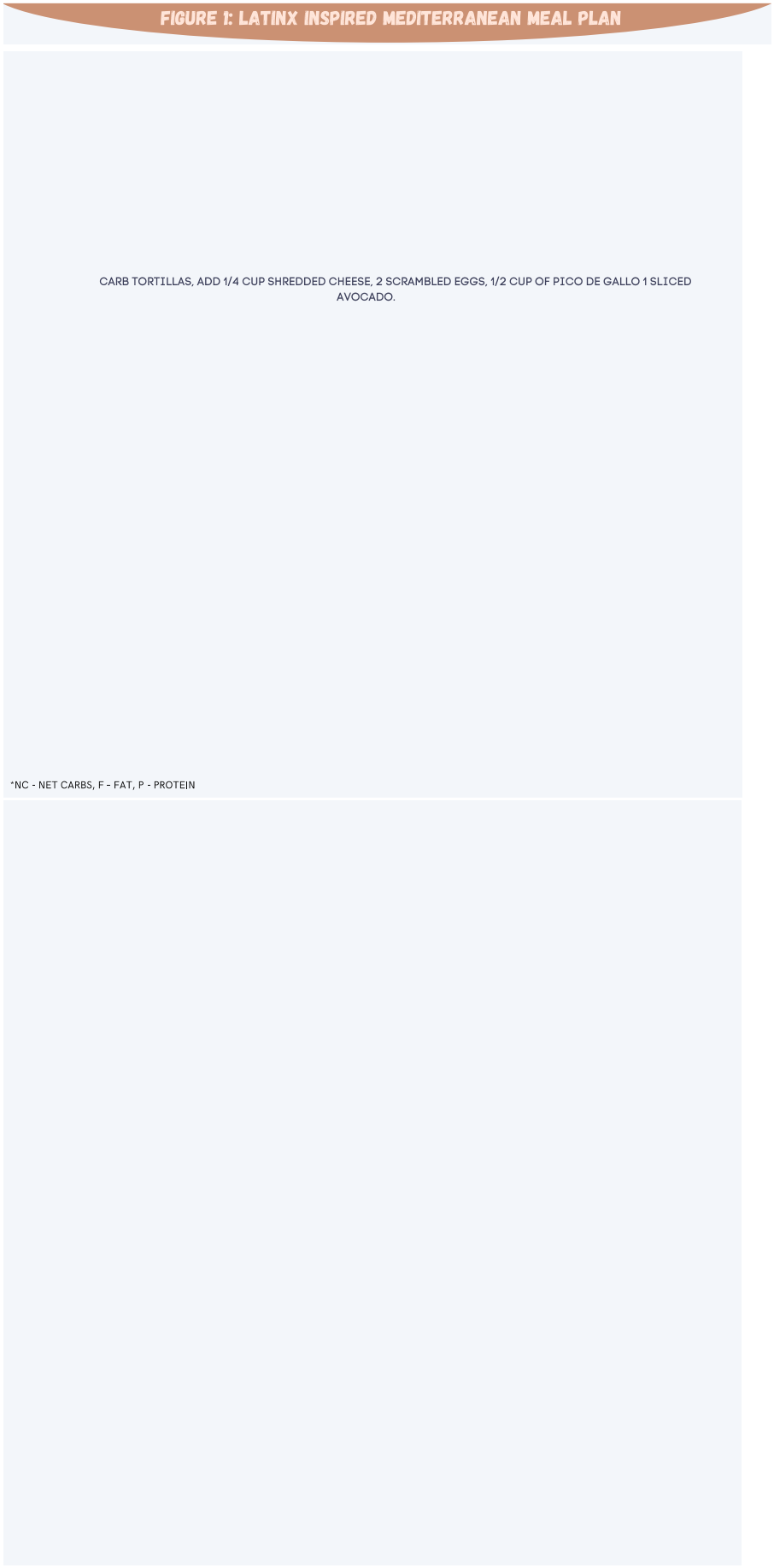


FIGURE 1: LATINX INSPIRED MEDITERRANEAN MEAL PLAN
CARB TORTILLAS, ADD 1/4 CUP SHREDDED CHEESE, 2 SCRAMBLED EGGS, 1/2 CUP OF PICO DE GALLO 1 SLICED AVOCADO.
*NC - NET CARBS, F - FAT, P - PROTEIN

PO3-09
Staging chronic liver injury and fibrosis using the enzyme biomarkers circulating fibroblast activation protein alpha (cFAP) and cDPP4

Vincent Wang[1], Bobby Boumelhem[1], Hui Zhang[2], William Bachovchin[3], Jacob George[2], Mohammed Eslam[2], Geraldine Ooi[4], Leon Adams[5], Geoff McCaughan[1 6], Avik Majumdar[7 8], Mark Gorrell[1]
[1]The University of Sydney, Centenary Institute, Sydney, Australia, *[2]Westmead Institute for Medical Research, Westmead, Australia*, *[3]Tufts University, Biochemistry, Boston, United States*, *[4]Monash University, Surgery, Melbourne, Australia*, *[5]The University of Western Australia, Faculty of Medicine and Health Sciences, Perth, Australia*, *[6]Royal Prince Alfred Hospital, AW Morrow Gastroenterology and Liver Centre, Camperdown, Australia*, *[7]The Austin Hospital, Gastroenterology, Melbourne, Australia*, *[8]The University of Melbourne, Medicine, Melbourne, Australia*
Email: m.gorrell@centenary.org.au

Background and aims: Metabolic dysfunction associated fatty liver disease (MAFLD) is a complex, multifaceted disease spectrum that drives chronic liver injury leading to fibrosis, which is the major correlate of adverse health outcomes. Liver biopsy is invasive and subject to availability, affordability and sampling variability. Therefore, there is a clear need to develop accurate non-invasive tests (NITs) that address these issues. Such NITs must accurately discriminate severity of liver disease while minimising indeterminate results based on an algorithm that incorporates liver biomarkers. We previously showed that fibroblast activation protein (FAP) is a gelatinase and is strongly expressed in the collagen regulating cells, the myofibroblasts and activated stellate cells, in human cirrhosis. Dipeptidyl peptidase 4 (DPP4) is a therapeutic target in diabetes mellitus. We have shown that DPP4 is relocated from apical to basolateral domains of hepatocytes in cirrhosis and that cDPP4 correlates with transaminase levels in MAFLD. Thus, we aimed to investigate the utility of cFAP and cDPP4 as biomarkers of fibrosis and steatosis, respectively, in MAFLD.

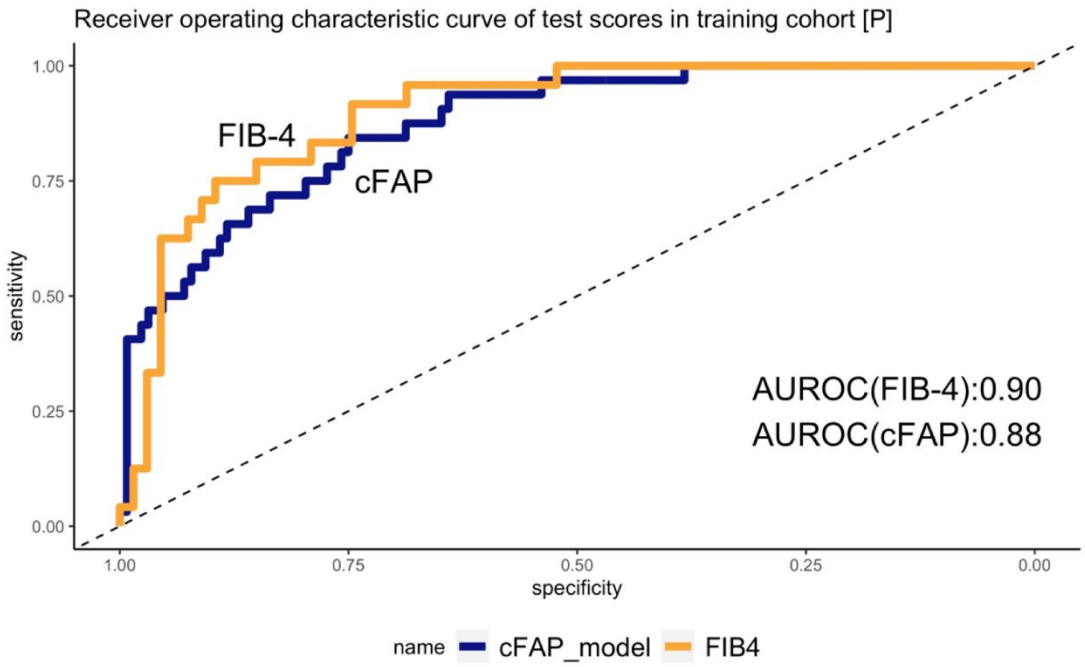
Method: Both of these proteins are shed from their respective cell surfaces as active enzymes that are relatively stable in human serum and plasma. In-house enzyme assays that are specific for each protease were applied to sera from two bariatric cohorts (training cohort P, n = 160, 36% diabetic; n = 182, 23% diabetic) and a MAFLD cohort (n = 150).

Results: Insulin resistance associated with cFAP in the MAFLD cohort ($p = 0.007$). A multi-variate model was developed combining cFAP with age, diabetes status and ALT to evaluate individuals who had an indeterminate diagnosis regarding liver fibrosis following an established NIT. On the training cohort, the AUROC was 0.875 (Figure) with a negative prediction value of 92% and a positive prediction value of 95%, indicating high accuracy. By serial application of the cFAP model then an established NIT, the number of indeterminate results approximately halved, to <15%. In both bariatric cohorts, steatosis grades 2 and 3 were associated with elevated cDPP4, with the association strengthened when adjusted for diabetes status, ALP and AST:ALT.

Conclusion: We present novel, inexpensive diagnostic tools for assessing whether an individual has severe MAFLD; using cFAP for fibrosis and cDPP4 for steatosis. The cDPP4 level very probably reflects hepatocyte damage, but might also in part reflect metabolic status. We suggest that an advantage of including cFAP in clinical fibrosis assessments is that FAP is strongly expressed by liver

cell types that regulate the extracellular matrix.

Figure:



PO3-10-YI
Serum protein induced by vitamin k absence or antagonist II for the prediction and risk stratification of HCC development in patients with metabolic dysfunction-associated steatotic liver disease on long-term follow-up

Gian Paolo Caviglia[1], Chiara Rosso[1], Marta Guariglia[1], Kamela Gjini[1], Amina Abdulle[1], Angelo Armandi[1 2], Daphne D'Amato[1], Gabriele Castelnuovo[1], Nuria Pérez Diaz del Campo[1], Maria Lorena Abate[1], Antonella Olivero[1], Giorgio Maria Saracco[1 3], Elisabetta Bugianesi[1 3]
[1]University of Turin, Department of medical science, Turin, Italy, *[2]University medical center of the Johannes Gutenberg-University, I.Department of medicine, Mainz, Germany*, *[3]Città della salute e della scienza- Molinette Hospital, Gastroenterology Unit, Turin, Italy*
Email: gianpaolo.caviglia@unito.it

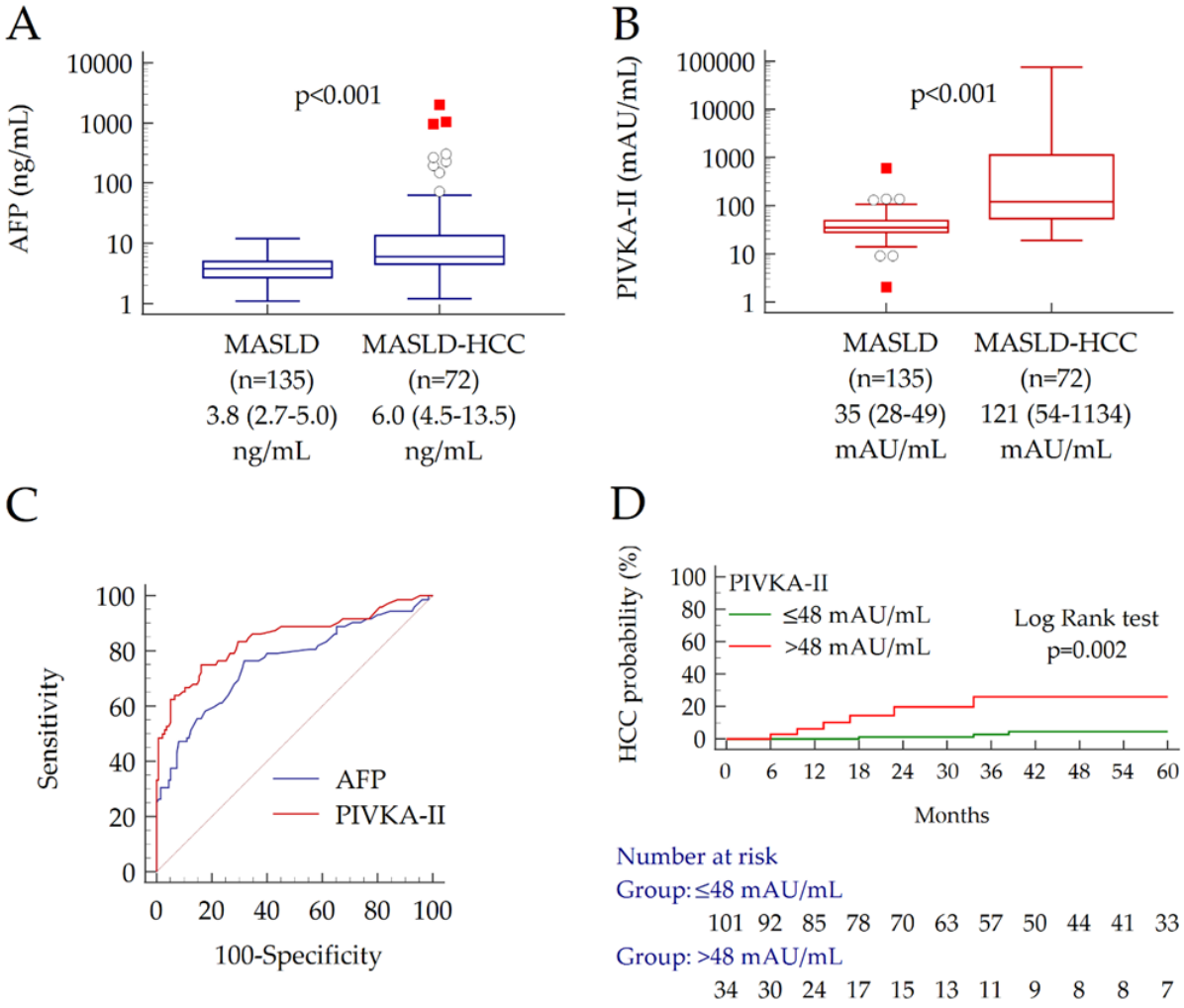
Background and aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) is projected to become the leading cause of hepatocellular carcinoma (HCC) worldwide. Currently, the surveillance of patients at risk of HCC development is based on a one-size-fits-all approach (i.e. semestral US ± α-fetoprotein [AFP]); the identification of reliable tools for the implementation of a risk-based surveillance is an unmet medical need. The aim of the study was to 1) investigate the diagnostic accuracy of protein induced by vitamin K absence or antagonist II (PIVKA-II) in comparison to AFP for HCC detection, and 2) to assess the performance of PIVKA-II for the stratification of the risk of HCC development in patients with MASLD on long-term follow-up (FU).

Method: We retrospectively enrolled 72 patients with MASLD-related HCC (age: 67 [62-70] years; 57 males [79.2%]; BMI: 29.1 [25.9-31.7] kg/m^2; T2DM: 43 [59.7%]; liver cirrhosis: 63 [87.5%]) and 135 consecutive patients with biopsy-proven MASLD or clinical diagnosis of MASLD-related liver cirrhosis without HCC (age: 58 [50-66] years; males: 65 [48.1%]; BMI: 31.0 [28.0-33.2] kg/m^2; T2DM: 78 [57.8%]; F3 = 55, F4 = 80). All the 135 patients without HCC had at least 6 months FU with regular US surveillance. Serum AFP and PIVKA-II were measured by chemiluminescent immunoassay on Lumipulse® G600 II platform (Fujirebio Inc., Tokyo, Japan).

Results: Median AFP and PIVKA-II serum values were significantly different between patients with HCC and those without (Figure A-B). PIVKA-II showed a superior performance for HCC detection as compared to AFP (AUC = 0.85 vs. 0.76; DeLong test: p = 0.053; Figure C). Patients without HCC at baseline (n = 135) were followed for a median of 3.1 (1.4-6.0) years; during the FU, 12 (8.9%) patients developed HCC (incidence rate: 2.08 per 100 person/year). Only 1 HCC occurred in F3 patients (incidence rate: 0.35 per 100 person/years), while 11 HCC occurred in patients with liver cirrhosis (incidence rate: 3.72 per 100 person/years) (p = 0.027). Overall, the performance of AFP and PIVKA-II for HCC prediction was C-index = 0.68 and 0.73, while in patients with cirrhosis the predictiveness of PIVKA-II outperformed AFP (C-index = 0.74 vs. 0.60). With a cut-off = 48 mAU/ml (Youden index), PIVKA-II provided a significant patients' stratification according to the individual risk of HCC development (p = 0.002; Figure D); notably, no HCC occurred in patients with baseline PIVKA-II ≤48 mAU/ml during the first 18 months of FU. At multivariate analysis corrected for age (HR = 1.02; p = 0.646), gender (HR = 0.90; p = 0.860), and cirrhosis (HR = 10.71; p = 0.037), PIVKA-II >48 mAU/ml resulted significantly and independently associated with HCC development (HR = 6.56; p = 0.002).

Conclusion: Serum PIVKA-II was superior to AFP for the detection of MASLD-related HCC. The measurement of PIVKA-II may be useful to tailor personalized surveillance strategies in patients with MASLD and advanced liver disease.

Figure:


A
AFP (ng/mL)
10000
1000
100
10
1
p<0.001
MASLD
(n=135)
3.8 (2.7-5.0)
ng/mL
MASLD-HCC
(n=72)
6.0 (4.5-13.5)
ng/mL
B
PIVKA-II (mAU/mL)
100000
10000
1000
100
10
1
p<0.001
MASLD
(n=135)
35 (28-49)
mAU/mL
MASLD-HCC
(n=72)
121 (54-1134)
mAU/mL
C
Sensitivity
100
80
60
40
20
0
AFP
PIVKA-II
0 20 40 60 80 100
100-Specificity
D
HCC probability (%)
100
80
60
40
20
0
PIVKA-II
≤48 mAU/mL
>48 mAU/mL
Log Rank test
p=0.002
0 6 12 18 24 30 36 42 48 54 60
Months
Number at risk
Group: ≤48 mAU/mL
101 92 85 78 70 63 57 50 44 41 33
Group: >48 mAU/mL
34 30 24 17 15 13 11 9 8 8 7

PO3-11
Alcohol overconsumption is common in MASLD and highly increases risk for future cirrhosis

Patrik Nasr[1 2], Axel Wester[2], Mattias Ekstedt[1], Rickard Strandberg[2], Stergios Kechagias[1], Ying Shang[2], Linnea Widman[2], Hannes Hagström[2]
[1]Linköping University Hospital, Sweden, *[2]Karolinska Institute, Sweden*
Email: hannes.hagstrom@ki.se

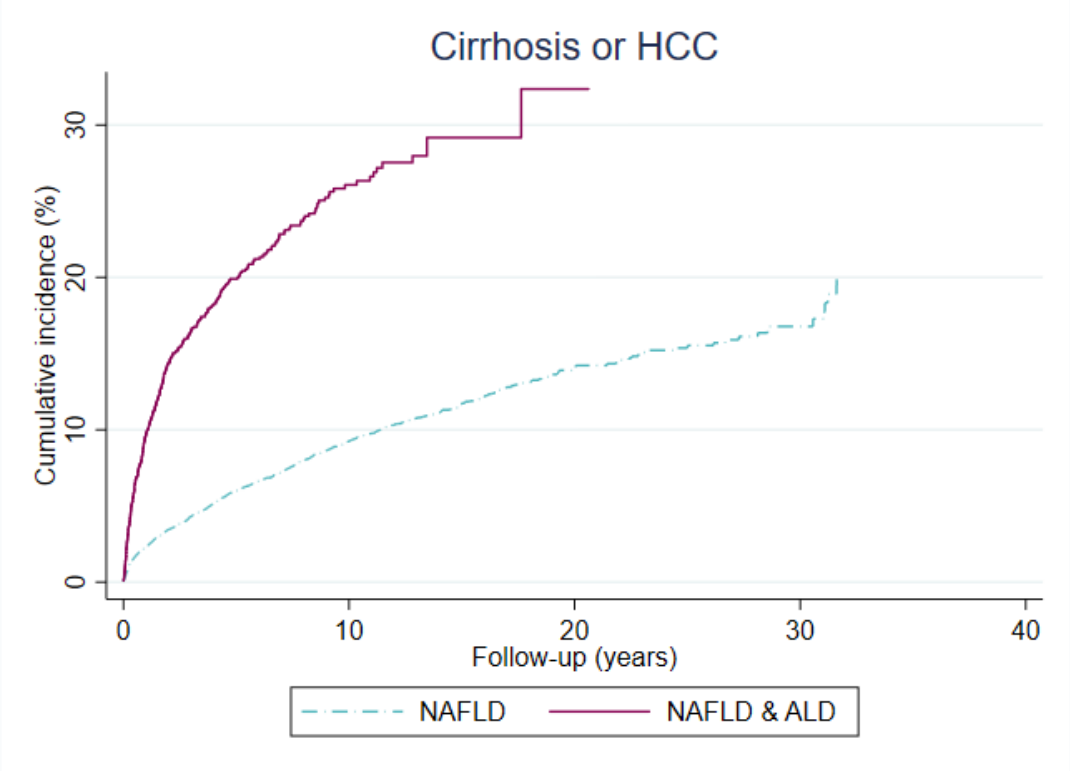
Background and aims: Alcohol overconsumption is a risk factor for disease progression in patients with metabolic-dysfunction associated steatotic liver disease (MASLD). How commonly this occurs and how it affects progression to major adverse liver outcomes (MALO) is not well known.

Method: We did a register-based cohort study, including all patients with a diagnosis of MASLD in Sweden between 1987 and 2020, without cirrhosis. Patients were stratified on co-occurrence of diagnoses of alcohol-related liver disease (ALD) or alcohol use disorder (AUD) prior to MASLD diagnosis. Incident MALO:s, defined as diagnoses of compensated or decompensated cirrhosis or HCC were derived from national registers. Cox regression was used to calculate hazard ratios for incident MALO.

Results: A total of 15, 107 patients with MASLD were identified. Median age was 55 years and 52% were female. 1, 843 (12%) had a prior diagnosis of ALD or AUD. During follow-up, a further 787 (5%) had a diagnosis of ALD or AUD. Patients with prevalent ALD or AUD diagnoses had considerably higher rates of MALO compared to patients without (19.5% vs 7.8%, aHR = 3.12, 95%CI = 2.74-3.55). The cumulative incidence of MALO was 9.2% in patients with only MASLD, and 25.8% in patients with both MASLD and ALD or AUD. Acquiring an ALD or AUD diagnosis after baseline was associated with higher rates of MALO (aHR = 5.81, 95%CI = 4.90-6.88).

Conclusion: ALD or AUD is commonly diagnosed prior to or after MASLD diagnosis. Such patients have considerably higher rates of progression to MALO. These data suggest misclassification is common in patients with MASLD. Alcohol history is vital to assess prognosis.

Figure:



PO3-12
Traditional indigenous land-based diet and non-alcoholic fatty liver disease

Julia Uhanova[1], Gerald Minuk[1], Byron Beardy[2], Jacquelin Linklater[2], Larry Wood[2], Adebanke Oketola[1], Daria Surina[1]
[1]University of Manitoba, Internal Medicine, Winnipeg, Canada, *[2]Four Arrows Regional Health Authority, Food Security, Winnipeg, Canada*
Email: julia.uhanova@umanitoba.ca

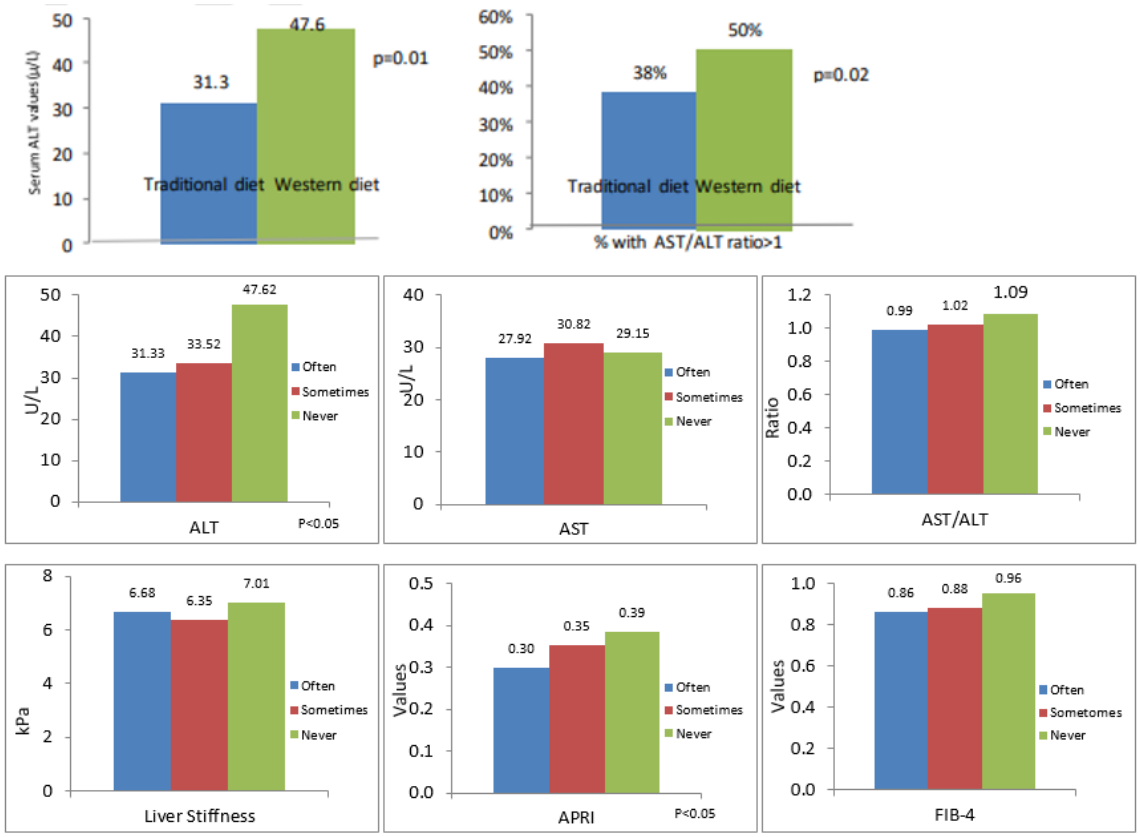
Background and aims: NAFLD is the most common liver disease, affecting 25-30% of the world's population and causing substantial morbidity and mortality. Analyzing 25 years of referral patterns to our Winnipeg HSC Hepatology clinic, we documented a significant increase in referrals for NAFLD, from 4.5% in 1992 to 60% in 2017. However, there were no data regarding how common or severe NAFLD is in Canadian Indigenous communities. Hence, our 5-year CIHR-funded study "Non-Alcoholic Fatty Liver Disease (NAFLD): Defining the impact, severity and natural history of NAFLD in Canadian First-Nations and non-First Nations communities" is designed to examine the NAFLD burden, short-term outcomes and contributing factors among community based First Nations (FN) and non-FN Manitobans. The principal aim of this study is to determine whether the extent of traditional FN dietary intake as determined by self-report correlates with the extent of hepatic inflammation and fibrosis in NAFLD patients.

Method: As part of the main study, 841 residents of Island Lake Anishininew Nations completed a detailed questionnaire of traditional indigenous diet consumption, with each item quantified as consumed "often" (on a daily to weekly basis), "sometimes" (several times a month) or "never" (consumed a few times per year or not at all). Laboratory parameters and markers of inflammation among the participants were analyzed with regard to the self-reported frequency of a traditional indigenous food consumption.

Results: In the dietary survey of 841 Anishininew First Nations, approximately 30% self-reported adhering to a traditional FN diet. Those who regularly consumed traditional FN food had significantly lower ALT (31.3 vs 47.6, $p < 0.01$) compared to those who did not regularly used traditional foods. The proportion of persons with the AST/ALT ratio>1, a reflection of disease severity, was also significantly lower in those who adhered to traditional FN diet than in individuals never/rarely consuming the Traditional FN diet (38% vs. 50%, $p < 0.02$). Markers of fibrosis (APRI and FIB-4) from subjects who reports adhering to the traditional Indigenous pattern of diet differed from those who do not consume Traditional food on a regular basis. While not all statistically significant, the similarities in trends in ALT, AST/ALT, APRI and Fib-4 were intriguing and need further investigation (the study is ongoing).

Conclusion: Persons reporting adherence to a traditional FN diet had significantly lower markers of hepatic inflammation. The non-invasive markers of fibrosis (APRI, FIB-4), while not statistically significant, had a similar tendency to increase with the decreased consumption of traditional food.

Figure: Laboratory parameters and markers of inflammation and fibrosis among the participants who self-reported consumption of a Traditional FN diet: often, sometimes, or never.



PO3-14
Associations of the fibrosis-4 index with rates of cardiac events and liver decompensation, and the potential implications on risk stratification in patients with non-alcoholic steatohepatitis

Lars Hegstrom[1], Estaban Gnass[1], Pete Vu[2], Ediz Calay[1], Tyler Wagner[1], Jesse Fishman[2]
[1]nference, Cambridge, United States, *[2]Madrigal Pharmaceuticals, Conshohocken, United States*
Email: lhegstrom@nference.net

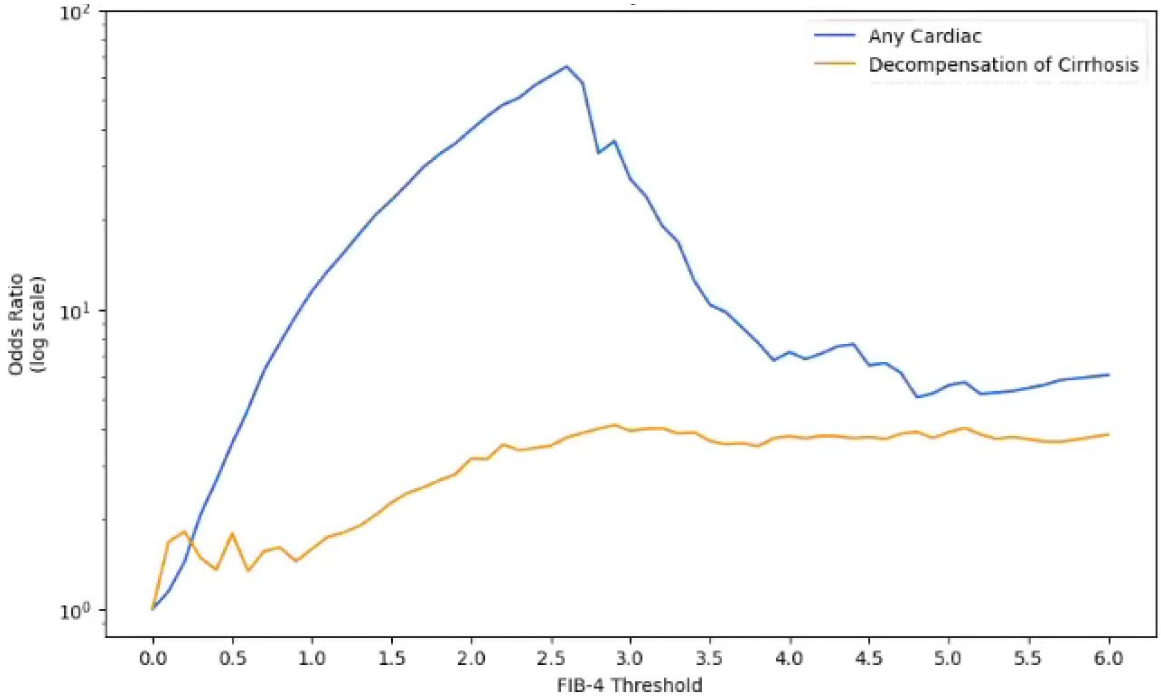
Background and aims: The non-invasive Fibrosis-4 index (FIB-4) predicts liver fibrosis and cirrhosis in patients. FIB-4 as the primary metric for risk stratification in non-alcoholic steatohepatitis (NASH) presents several critical limitations, notably regarding the risk of cardiovascular (CV) events. Recent studies highlight the FIB-4's potential in forecasting cardiac events, however it is critical to note that FIB-4 cutoffs as currently applied may not precisely represent the risk for these outcomes. This study aims to examine association between CV events and FIB-4 score.

Method: Our study cohort comprised 1, 828 biopsy-confirmed NASH patients extracted from a de-identified, privacy-preserving database consisting of digital electronic health records (EHR) from approximately 7 million patients seen at sites over five different states from 1991 through 2020. Patients with coexisting conditions, such as hepatocellular carcinoma, alcoholism, elevated HgA1c >9% or ALT >250 U/L, viral encephalitis, bariatric surgery, and autoimmune disease, were excluded to maintain a clear focus on NASH-specific outcomes. The identification and selection of eligible patients employed a hybrid approach beginning with structured ICD diagnosis that was then enriched by natural language processing algorithms.

Results: Analysis revealed patterns in the relationship between FIB-4 scores and the odds ratios of liver decompensation and CV events (myocardial infarction, congestive heart failure, unstable angina, cardiac arrest, and aneurysm dissection). For CV outcomes, the odds ratio is associated with a FIB-4 score up to 2.60, and up to 2.90 for liver decompensation. The peak FIB-4 values of 2.60 and 2.90, at which point patients are most likely to experience an undesirable outcome, are close to the guideline-recommended FIB-4 cutoff of >2.67. We observed a steep decrease in the odds ratio for cardiac outcomes at FIB-4 >2.60, while the odds ratio for liver outcomes remained steady, reinforcing a shift in the relative incidences of outcomes experienced by patients with advanced liver disease. Of note with a FIB-4 score as low as 1.0, the risk for any cardiac event is significantly elevated. This is lower than the guideline-recommended FIB-4 minimum of 1.3.

Conclusion: These findings underscore the capacity of FIB-4 to forecast not only liver decompensation but also CV events in patients with NASH. The peak odds for CV events is approximately 2.60, and 2.90 for decompensation to cirrhosis, with significant risk for CV events as low as FIB-4 score of 1.0. Such findings support the need to stratify patients with an elevated FIB-4 as high-risk and also underline the critical need to revisit the lower thresholds of FIB-4 scores, now in light of considering the predictive capacity for cardiac events.

Figure: Odds ratio by FIB-4 score



PO3-16
Serum free fatty acids in children with metabolic-associated fatty liver fibrosis

Nataliya Zavhorodnia[1], Inna Klenina[2], Oksana Tatatrchuk[2], Olena Hrabovskaya[2]
[1]SI "Institute Gastroenterology of the National Academy of Medical Sciences of Ukraine, Pediatric Gastroenterology Department, Dnipro, Ukraine, *[2]SI "Institute Gastroenterology of the National Academy of Medical Sciences of Ukraine, Scientific Research Department, Dnipro, Ukraine*
Email: nzavgorodni75@gmail.com

Background and aims: Metabolic dysfunction-associated fatty liver disease (MAFLD) is the most common chronic liver disease affecting the pediatric population and is closely linked to insulin resistance and altered lipid metabolism. MAFLD is associated with long-term outcomes such as cardiovascular and end-stage liver diseases. Lipid abnormalities may contribute to disease progression, especially in patients with a higher fibrosis stage. So, the aim of our study was to investigate the changes in serum free fatty acids content in MAFLD patients depending on the presence of liver fibrosis.

Method: 80 obese children were included in the study. According to transient elastography data (Fibroscan502touch, Echosense, France), obese patients were divided into 3 groups: 1 group consisted of 27 MAFLD children with fibrosis ≥F1, 2 group-35 MAFLD children without fibrosis, 3 group- 18 obese children without MAFLD and fibrosis. The control group (4 group) consisted of 14 children with normal weight without MAFLD and fibrosis. Serum FFA fractions were investigated with gas chromatography using "Chromatek-Crystal 5000. Total FFA, saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), unsaturated fatty acids (UFA), and monounsaturated fatty acids (MUFA) content were determined. The atherogenicity index of FFA (AI_{FFA}) and stearic to oleic fatty acid ratio (k1 C18:0/C18:1) were calculated.

Results: A significant decrease in the total serum FFA content was found: in 1 group children-by 1.7 times ($p < 0.05$), in 2 group-by 1.9 times ($p < 0.05$), in 3 group-by 2.1 times ($p < 0.05$) compared to 4 group. The MUFA content was decreased in all groups: in 1 group by 3.9 times ($p < 0.001$), in 2 group-by 2.3 times ($p < 0.01$), in 3 group-by 2.5 times ($p < 0.01$) compared to 4 group. The MUFA content in 1 group children was significantly lower than in 2 and 3 group children ($p < 0.05$). The median SFA content decreased in all groups: in 1 group-by 1.3 times ($p>0.05$), in 2 group-by 1.8 times ($p < 0.01$), in 3 group-by 1.3 times ($p>0.05$), compared to 4 group. The median level of serum UFA significantly decreased in all groups: in 1 group-by 1.9 times ($p < 0.05$); in 2 group-by 2.3 times ($p < 0.05$); in 3 group-by 2.4 times ($p < 0.01$) compared to 4 group. Similar changes were established for the serum PUFA content: a decrease in 1 group by 1.2 times, in 2 group-by 1.9 times ($p < 0.05$), in 3 group- by 2.2 times ($p < 0.05$) was found compared to 4 group. The k1 C18:0/C18:1 ratio was increased: in 1 group by 8.6 times ($p < 0.01$), in 2 group-by 4.0 times ($p < 0.01$), in3 group-by 1.5 times compared to 4 group. The median AI_{FFA} levels were decreased in all groups children: in 1 group-by 2.2 times ($p < 0.01$), in 2 group-by 1.6 times ($p < 0.01$), in 3 group-by 1.2 times compared to 4 group.

Conclusion: Thus, significant changes in serum FFA content were observed in children with metabolic-associated fatty liver fibrosis due to a decrease of MUFA, AI_{FFA}, and an increase of k1 C18:0/C18:1 ratio which needs further evaluation as liver fibrosis markers.

PO3-17-YI
Transition from non-alcoholic fatty liver disease to steatotic live disease: real-world evidence in a cohort of individuals with type 1 and type 2 diabetes

Jonathan Mertens[1 2], Tammy Huybrechts[2 3], Luisa Vonghia[2 4], Eveline Dirinck[1 2], Wilhelmus Kwanten[2 4], Wim Van Hul[2 3], Christophe De Block[1 2], Sven Francque[2 4]
[1]Antwerp University Hospital, Endocrinology, Diabetology and Metabolism, Edegem, *[2]University of Antwerp, Laboratory of Experimental Medicine and Paediatrics, Antwerpen, Belgium*, *[3]Centrum Medische Genetica Universitair Ziekenhuis Antwerpen, Edegem, Belgium*, *[4]Antwerp University Hospital, Gastroenterology and Hepatology, Edegem*
Email: jonathan.mertens@student.uantwerpen.be

Background and aims: A consensus was reached to change the nomenclature and diagnostic criteria of non-alcoholic fatty liver disease (NAFLD) towards the umbrella term steatotic liver disease (SLD), with the inclusion of several sub-categories, based on the presence of cardiometabolic risk factors (Metabolic Dysfunction Associated SLD, MASLD), increased alcohol consumption (metALD) and co-existence of other liver disease. Real-world data are missing in risk groups to determine the cross-over between the new and old nomenclature criteria. This study aims to examine the diagnostic agreement between both definitions in a cohort of people with diabetes.

Method: This is a retrospective analysis of a tertiary care centre cohort with either type 1 diabetes (T1D), insulin-naïve type 2 diabetes (T2D-IN) or insulin-dependent type 2 diabetes (T2D-ID) prospectively screened for liver steatosis using abdominal ultrasound. Individuals were consecutively invited during their annual check-up. Only inclusion criterion was having diabetes. All participants were screened for other liver disease (viral hepatitis, monogenic liver disease, use of steatogenic drugs) and had to fill out a standardized questionnaire to estimate daily alcohol consumption. Diagnosis was based on previous NAFLD guidelines or the new SLD criteria. Prevalence rates where compared. We did not use the cardiometabolic criterion of hyperglycaemia/elevated HbA1c/antidiabetic treatment in subjects with T1D, due to their auto-immune aetiology of hyperglycaemia.

Results: A total of 1177 individuals were included: 661 patients with T1D, 248 with T2D-IN, and 268 with T2D-ID. Steatosis was present in 118/661 individuals with T1D (17.9%). Based on the criteria for NAFLD, 109 individuals had NAFLD (16.5% of total, 92.4% of people with steatosis). According to the new criteria, 109 subjects had MASLD, while 5 had metALD (0.8%). Four subjects (0.3%) had a combination of MASLD with another cause other than alcohol (steatogenic drugs in all 4). In those with T2D-IN, steatosis was present in 159/248 individuals (64.1%). NAFLD was present in 151 individuals (60.9% of total, 95.0% of people with steatosis). MASLD was present in 151 subjects (60.9%), while 5 subjects had metALD (2.0%), and 4 (1.6%) had MASLD combined with another aetiology (steatogenic drugs n = 3, hepatitis B n = 1). In people with T2D-ID, 171/268 had steatosis (63.8%). NAFLD was present in 150 subjects (56.0% of total, 87.8% of those with steatosis). MASLD was present in 150 cases (56.0%). MetALD was present in 10 subjects (3.7% of cases), 11 people (4.1%) had MASLD combined with another aetiology (steatogenic drugs n = 4, viral hepatitis n = 6, alfa-1 antitrypsin deficiency n = 1). The general low prevalence of metALD could be explained partially due to participation bias.

Conclusion: Liver steatosis is common in people with T1D, and highly prevalent in people with T2D, regardless of the use of insulin. Agreement between NAFLD and MASLD was 100% in subjects with T1D, T2D-ID, or T2D-IN.

PO3-18
The effect of dapagliflozin on fibrosis and inflammation in patients with metabolic dysfunction-associated steatohepatitis

Elena Laura Iliescu[1 2], Letitia Toma[1 2], Ioana Tanasie[2], Adriana Mercan-Stanciu[2], Razvan Rababoc[2]
[1]Fundeni Clinical Institute, Internal Medicine, Bucharest, Romania, *[2]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*
Email: laura_ate@yahoo.com

Background and aims: Dapagliflozin was first introduced as an antidiabetic medication, but its effects in cardiovascular and renal protection have been demonstrated. Recently, evidence has emerged regarding its beneficial effects in patients with metabolic dysfunction-associated steatotic liver disease. This study aims to evaluate the mid-term impact of dapagliflozin in reducing fibrosis and inflammation in patients with metabolic dysfunction-associated steatohepatitis (MASH).

Method: We performed a prospective clinical study including 138 consecutive patients diagnosed with MASH between June 2022 and January 2023, among which 74 (53.6%) were diabetic. We excluded patients with other causes of liver disease, liver nodules, or active infections. All patients were given 10mg of dapagliflozin daily. Patients were evaluated for fibrosis and inflammation at the beginning of the treatment and at 6 months. Evaluation for fibrosis included Fibroscan®, Fib-4 and Fibrotest; inflammation was evaluated by serum levels of transaminases, C reactive protein (CRP), interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α).

Results: The mean age in the study lot was 56.34 ± 14.74 years, without significant difference between diabetic and non-diabetic patients. Initial fibrosis evaluation revealed 28 F1 patients (13.04%), 51 F2 patients (38.05%) 52 F3 patients (38.88%) and 7 F4 patients (5.22%). After therapy, serum levels of ALT, AST, IFN-γ and TNF-α decreased significantly both on diabetic and non-diabetic patients (Table 1). Also, mean values of liver stiffness (Fibroscan), Fibrotest and Fib-4 score decreased in all fibrosis groups (F1 to F3), except F4. Stiffness decreased from 5.8 to 2.6 (F1, p = 0.04), from 9.4 to 6.2 (F2, p = 0.01), from 12.6 to 9.3 (F3, p = 0.03). Fib 4 score decreased from 1.05 to 0.68 (F1, p = 0.03), 2.89 to 1.90 (F2, p < 0.01) 3.68 to 3.18 (F3, p = 0.04). Fibrotest decreased from 0.29 to 0.18 (F1, p = 0.02), 0.52 to 0.44 (F2, p = 0.01), 0.62 to 0.51 (F3).

Conclusion: Dapagliflozin treatment helps reduce inflammation and fibrosis in MASH patients, more significantly in those with moderate and severe fibrosis, regardless of the presence of diabetes

Figure: Table 1. Mean values for inflammatory parameters in diabetic and non-diabetic patients

	Diabetic patients N = 74		P value	Non- diabetic patients N = 64		P value
	Before therapy	At 6 months		Before therapy	At 6 months	
AST (N: 0-34 U/L)	87.28	36.62	0.01	75.16	31.63	0.01
ALT (N: 0-44 U/L)	93.31	38.19	0.01	94.73	34.42	0.01
CRP (N: 0-3 mg/L)	5.62	2.73	0.04	4.37	3.85	0.06
IFN-γ (N <0.1 UI/ml[6])	1.35	0.31	0.02	0.98	0.18	0.01
TNF-α (N <8.1pg/ml[2])	16.82	9.46	0.01	14.29	7.25	0.01

PO3-19-YI
High tyrosine and PLIN2 and low beta-hydroxybutyrate characterize subjects with metabolic dysfunction-associated steatohepatitis (MASH)

Patrizia Infelise[1 2], Silvia Sabatini[1], Giulia Angelini[3], Fabrizia Carli[1 2], Samantha Pezzica[1 2], Ornella Verrastro[3], Erminia Lembo[3], Geltrude Mingrone[3 4], Amalia Gastaldelli[1]
[1]Institute of Clinical Physiology, National Research Council, CNR, Pisa, Italy, *[2]Università degli Studi di Siena, Siena, Italy*, *[3]Università Cattolica del Sacro Cuore and Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Department of Medical and Surgical Sciences, Roma, Italy*, *[4]School of Cardiovascular and Metabolic Medicine and Sciences, King's College London, Division of Diabetes and Nutritional Sciences, London, United Kingdom*
Email: patriziainfelise@gmail.com

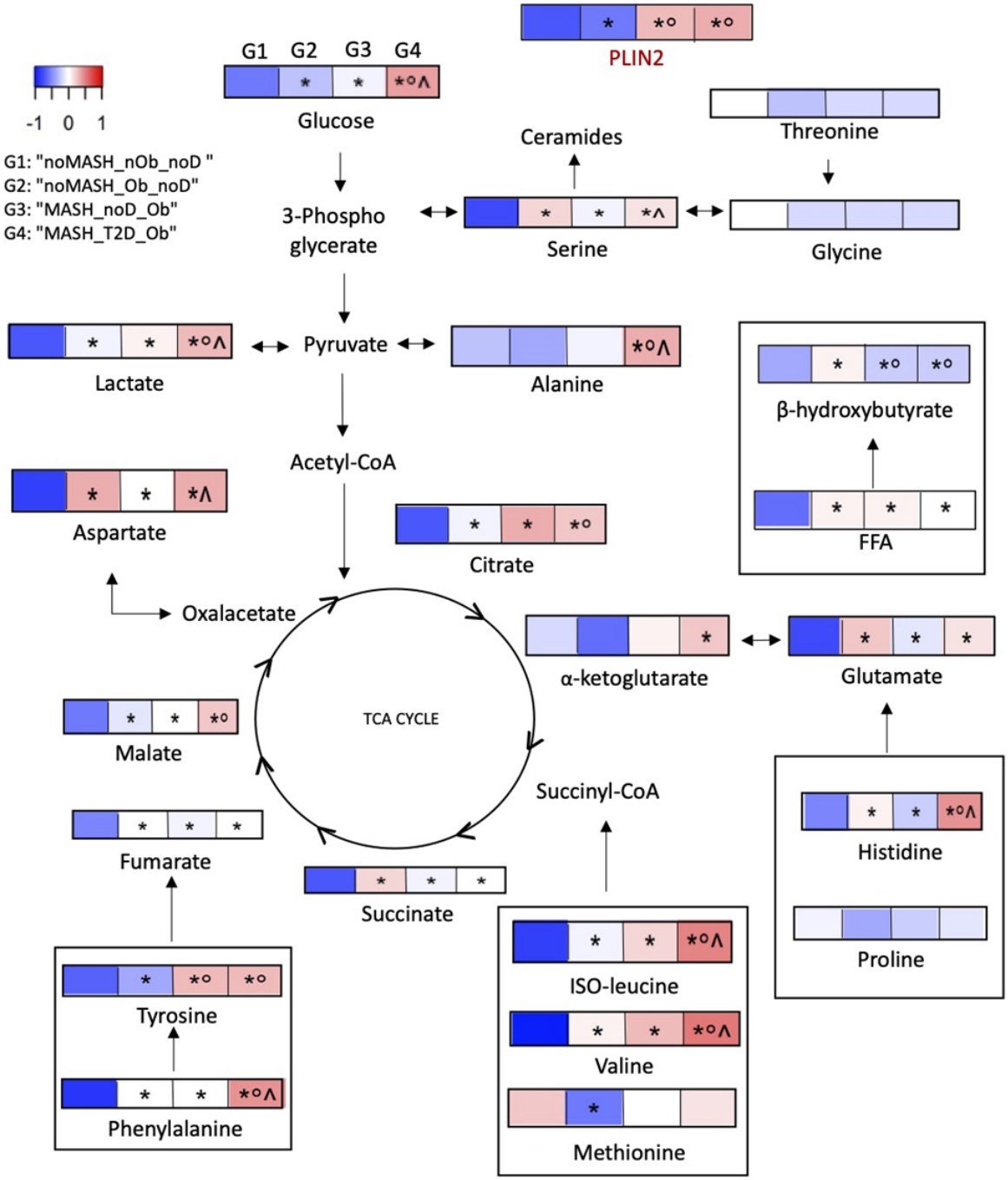
Background and aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) and steatohepatitis (MASH) are highly prevalent with comorbidities like obesity and type 2 diabetes (T2D). Metabolomics has been used to identify metabolites associated with metabolic dysfunction, but little is known in MASLD where the focus was mainly on lipidomics. Our aim was to identify which metabolites can discriminate MASH vs noMASH and study if T2D impacts on metabolomic profile.

Method: We analyzed the BRAVES cohort, 195 subjects with liver biopsy with noMASH (n = 78) or MASH (n = 117) and different histological response. To understand the impact of metabolic dysfunction on the phenotype noMASH and MASH the cohort was divided according to presence of if obesity (BMI ≥ 30) or T2D: G1 = noMASH_nOb_noD (n = 52 BMI <30, no MASH no T2D); G2 = noMASH_Ob_noD (n = 26 BMI ≥ 30, no MASH no T2D); G3 = MASH_noD_Ob (n = 55 BMI ≥ 30, with MASH no T2D); G4 = MASH_T2D_Ob (n = 62 BMI ≥ 30, with MASH and T2D). Target metabolomics was performed on plasma samples and polar metabolites, i.e., amino acids (AA) and organic acids related to TCA cycle, were quantified by mass spectrometry using labeled internal standards (^{13}C-^{15}N). We also measured FFA, TG, CHO, HDL, LDL, liver enzymes, indexes of insulin resistance (IR) as HOMA-IR and ADIPO-IR and PLIN2 abundance in monocytes, recently shown to be a marker of MASH.

Results: The concentrations of AA like BCAAs, phenylalanine, tyrosine, glucogenic substrates (alanine, lactate, serine, glutamate), TCA cycle energy substrates (citrate, alpha-ketoglutarate, malate) and lipids (TG, CHO) were increased in subjects with obesity, further increased by the presence of MASH and T2D (figure). In G2-G4 succinate, fumarate, FFA and beta-hydroxybutyrate were higher than in G1, but the values decreased in MASH_noD_Ob and further in MASH_T2D_Ob. The metabolic profile of MASH_T2D_Ob was the worst. Fasting hyperglycemia was correlated with increased glucogenic substrates, IR and FFA that were high but beta-oxidation was reduced. Increased tyrosine, PLIN2 and decreased beta-hydroxybutyrate discriminated MASH vs noMASH, regardless of obesity and T2D. Moreover, PLIN2 was correlated with BCAAs, glucogenic and TCA cycle metabolites, FFA, ADIPO-IR, HOMA-IR, TG and Hb1Ac ($r > 0.18$, $p < 0.05$).

Conclusion: Subjects with MASH have increased concentrations of PLIN2, several glucogenic substrates, TCA cycle intermediates mainly related to presence of obesity while MASH and T2D was associated to decreased succinate and beta-oxidation. Only high tyrosine and PLIN2 and low beta-hydroxybutyrate were specifically associated to MASH regardless of T2D.

Figure:



Metabolites of interest and their changes across the groups. Median within the groups is reported. Data were mean-centered and scaled to standard deviation equal to 1.
*p < 0.05 vs G1; °p < 0.05 vs G2; ^p < 0.05 vs G3.

PO4-01-YI
Design and optimisation of an automated decision support tool for diagnosing and treating metabolic dysfunction-associated steatotic liver disease in primary care patients with type 2 diabetes mellitus-a novel, algorithm-based approach

Damien Leith[1 2], YeunYi Lin[1 2], Paul Brennan[1 2], Ewan Pearson[1 2], John Dillon[1 2]
[1]*School of Medicine-University of Dundee, United Kingdom*, [2]*Ninewells Hospital, United Kingdom*
Email: damienleith86@gmail.com

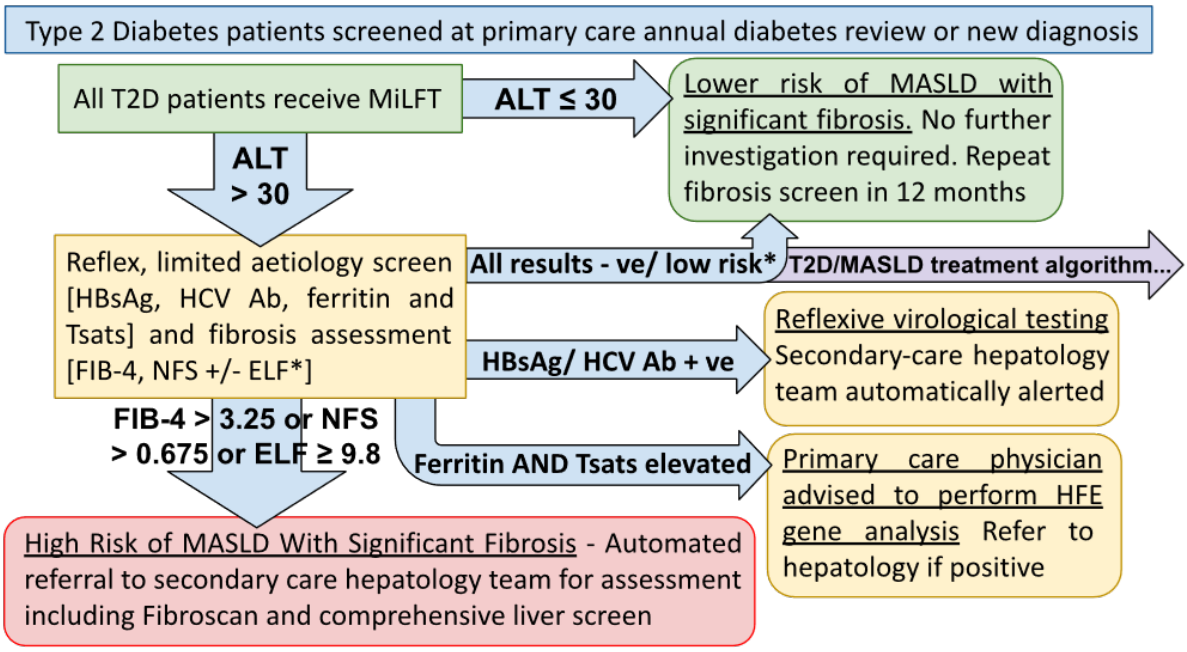
Background and aims: The prevalence of Metabolic Dysfunction-Associated Steatotic Liver Disease [MASLD] in Type 2 Diabetes [T2D] exceeds 70%, with increased rates of MASLD progression and cirrhosis compared to non-T2D patients. Latest guidelines recommend screening all T2D patients for MASLD and liver fibrosis, however, implementing screening into standard diabetes care can be challenging.
We aimed to developed a novel, lab based diagnostic algorithm to be used in primary care, incorporating precision medicine approaches to address these challenges

Method: We reviewed national and international guidelines for the diagnosis and management of non-alcoholic fatty liver disease [NAFLD] in T2D patients (NICE 2016, EASL-EASD-EASO 2016). A minimum required dataset for diagnosis of NAFLD was established. The new MASLD definition was incorporated and a new minimum dataset for the diagnosis of MASLD was converted into a diagnostic flow algorithm. Expert clinicians' consensus on thresholds for fibrosis testing and treatment recommendations were incorporated to create a didactic diagnostic and treatment algorithm.

Results: The finalised MASLD diagnostic algorithm [see figure] was converted into a pseudocode format. The algorithm was tested using ground truth patients and an anonymised real patient dataset. The algorithm performed well, classifying all patients accurately according to pre-set diagnostic thresholds. Finally, the completed algorithm was coded into the lab information system [LIMS] and output advice integrated into existing clinician and patient accessible diabetes care platforms.

Conclusion: Identifying MASLD in patients with T2D is essential but ensuring appropriate universal screening, linkage to hepatology and evidence-based treatment remains a significant challenge. We have designed and optimised an innovative, lab integrated MASLD screening algorithm which we believe will improve the identification and treatment of MASLD in T2D patients. It allows population screening for MASLD in T2D and referral of those at high-risk for advanced fibrosis to hepatology services via an automated process. Evidence based, individualised MASLD treatment recommendations are generated and integrated into the diabetes care of those not requiring specialist input. The utility of the algorithm will now be tested in a large, real-world primary care cohort as part of an integrated "iDiabetes" platform.

Figure:



* **FIB-4 cutoff: low risk < 1.45; indeterminate risk 1.45 to 3.25; high risk > 3.25**
NFS cutoff: low risk < -1.455; indeterminate risk -1.455 to 0.675; high risk > 0.675
ELF test performed if Fib4 ≥ 1.45 OR NFS ≥ -1.455; ELF test high risk ≥ 9.8

T2D: Type 2 Diabetes Mellitus; MASLD: Metabolic associated steatotic liver disease; MiLFT: Modified intelligent Liver Function Test; ALT: alanine aminotransferase; HBsAg: Hepatitis B surface antigen; HCV Ab: Hepatitis C antibody; Tsats: Transferrin saturations; FIB-4: Fibrosis 4 score; NFS: NAFLD Fibrosis Score; ELF: Enhanced Liver Fibrosis test

PO4-02-YI
Diabetic advanced steatotic liver disease is a surrogate for the other complications of type 2 diabetes mellitus

Zülal İstemihan[1], Fatih Bektaş[2], Ali Emre Bardak[3], Cansu Kızıltaş[3], Gamze Kemeç[3], Ziya Imanov[1], Volkan Senkal[1], Kanan Nuriyev[1], Aynure Rüstemzade[1], Sezen Genç[1], Hülya Hacışahinoğulları[2], Kubilay Karşıdağ[2], Bilger Çavuş[1], Aslı Çifcibaşı Örmeci[1], Filiz Akyuz[1], Kadir Demir[1], Fatih Besisik[1], Sabahattin Kaymakoglu[1]
[1]Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Gastroenterohepatology, Istanbul, Turkey, *[2]Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolic Diseases, Istanbul, Turkey*, *[3]Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Istanbul, Turkey*
Email: kaymakoglus@hotmail.com

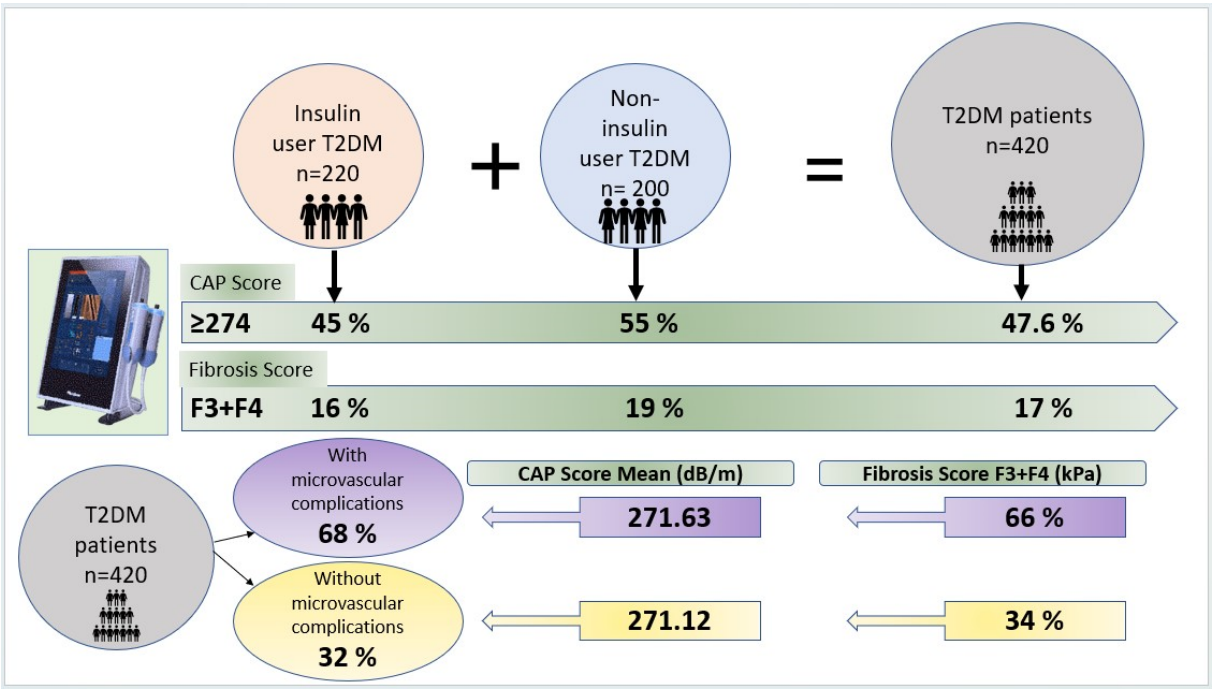
Background and aims: It was aimed to investigate the frequency of non-alcoholic fatty liver disease (NAFLD) with non-invasive tests and the association with insulin treatment and steatosis and liver fibrosis in type 2 diabetes mellitus (T2DM) patients.

Method: We prospectively evaluated the frequency of NAFLD and liver fibrosis with biomarkers based on blood tests and FibroScan® in adult (≥18 years) patients with T2DM and also we evaluated the association with insulin treatment and steatosis and liver fibrosis who were followed up from the T2DM outpatient clinic in a tertiary center. ≥8 kPa was accepted as significant fibrosis (F2), and ≥9.6 kPa was accepted as advanced fibrosis (F3-F4) in FibroScan® measurements. The cut-off value of the CAP score was accepted as 274 dB/m for steatosis. Fibrosis and steatosis were compared with FibroScan® and with scores based on blood tests.

Results: There were 420 T2DM patients (54.5% females) in the study. 52% of them were using insulin and oral antidiabetic agent (OAD), and others were just using OAD. The mean age of the patients was 60.59 ± 10.8 years, DM duration was 164.8 ± 107.8 months, body mass index (BMI) was 29.8 ± 5.8 kg/m^2. There was no significant difference to be overweight or obese between insulin users and non-users, but the obesity rate was significantly higher when insulin users were compared within themselves ($p = 0.033$). When the insulin users and non-users were compared, there was no significant difference between them in terms of age, BMI, and waist/hip ratio. Liver stiffness measurements (LSM) and CAP scores of the study group are summarized in Figure. While LSM was most significantly correlated with FIB4 and FAST scores ($p <0.01$, $r = 0.438$; $p <0.01$, $r = 0.670$, respectively), CAP score was correlated with fatty liver index and hepatic steatosis index ($p <0.01$, $r = 0.494$; $p <0.01$, $r = 0.508$, respectively). The frequency of NAFLD in T2DM was lower in insulin users than in non-users. (45%, 55%, respectively, $p = 0.004$). Insulin users had lower CAP scores ($p = 0.004$), and fibrosis values weren't different between the groups ($p = 0.26$). The FAST score was higher in those who did not use insulin ($p = 0.02$). There was no significant difference between the other scores. When insulin users were compared in terms of significant and advanced fibrosis rates, the incidence of advanced fibrosis was significantly higher ($p = 0.001$). Insulin users had higher HbA1c levels and worse DM controls ($p = 0.01$). Microvascular and macrovascular complications of T2DM were observed more frequently in patients using insulin ($p <0.05$). In addition, significant and advanced fibrosis was more common in T2DM patients with microvascular and macrovascular complications than in uncomplicated T2DM patients regardless of insulin use ($p = 0.001$).

Conclusion: NAFLD was detected more frequently in T2DM patients with non-using insulin than in users in our study population. Significant fibrosis was more common in patients with microvascular and macrovascular complications of T2DM.

Figure: LSM and CAP scores of the study group



PO4-03-YI
The PNPLA3 genotype of MASLD patients profoundly alters their metabolic profile even with a low BMI

Lina Jegodzinski[1], Ashok Rout[2], Darko Castven[1], Friedhelm Sayk[1], Franziska Schmelter[3], Bandik Föh[4], Henrike Dobbermann[1], Monika Herr[1], Sebastian Meyhöfer[1 5], Svenja Meyhöfer[1], Monika Rau[6], Susanne N Weber[7], Marcin Krawczyk[7], Andreas Geier[6], Ulrich Gunther[2], Jens Marquardt[1]
[1]University Hospital Schleswig-Holstein, Department of Medicine I, Lübeck, Germany, *[2]University of Lübeck, Institute of Chemistry and Metabolomics, Lübeck, Germany*, *[3]University of Lübeck, Institute of Nutritional Medicine, Lübeck, Germany*, *[4]Gemeinschaftspraxis im Gesellenhaus, Lübeck, Germany*, *[5]University of Lübeck, Institute for Endocrinology and Diabetes, Lübeck, Germany*, *[6]University Hospital Würzburg, Department of Medicine II, Würzburg, Germany*, *[7]University Hospital Saarland, Department of Medicine II, Homburg, Germany*
Email: lina.jegodzinski@uksh.de

Background and aims: Metabolic Dysfunction Associated Steatotic Liver Disease (MASLD) encompasses a heterogeneous spectrum of patients with different clinical and molecular characteristics. Pathophysiologically, obesity, diabetes and the metabolic syndrome are major exogenous determinants for metabolic liver diseases. In recent years, several common genetic variants have been identified with significant impact on MASLD development and progression. Herein, the *patatin*-like phospholipase domain-containing 3 (PNPLA3) p.I148M was identified as a major genetic risk factor for MASLD. While its impact on clinical progression is well established, metabolomic and lipidomic profile of *PNPLA3* variants are not fully understood.

Method: In this bi-centric study, a total of 248 MASLD patients were recruited. Chronic and acute liver disease other than MASLD were excluded in all patients. Genotyping of the *PNPLA3* p.I148M polymorphism was performed using TaqMan assays. Proteo-metabolomics of patient sera was performed by NMR spectroscopy, covering a range of ca. 30 metabolites as well as 100 lipoprotein and glycoprotein parameters.

Results: Homozygous *PNPLA3* p.148MM variant was present in 39 patients (15.7 %), whereas 86 patients (34.7 %) carried the p.148IM genotype. We were able to show clear differences in analysed serum metabolites as well as in lipoprotein subtype profiles between patients with different PNPLA3 genotypes. For example, there was a significantly higher proportion of LDL triglyceride and IDL as well as VLDL cholesterol subfractions in serum of patients carrying the p.148MM genotype. This phenotype was more pronounced in patients with a BMI <30 kg/m^2 than in obese individuals. Additionally, we observed differences in the amino acid status of the patients, especially in the levels of phenylalanine and tyrosine.

Conclusion: The *PNPLA3* p.I148M polymorphism has a profound and clinically relevant impact on metabolomic and lipidomic profil of MASLD patients. Interestingly, BMI only conferred to minor alterations in serum profiles underlining the importance of genotyping in distinct MASLD subgroups. These results might contribute to our understanding how the variant affects progression in MASLD patients. A detailed characterization of key molecules could provide a useful tool for the identification of patients at risk in the future.

PO4-04
Genetic markers of MASLD in an elderly Caucasian population and the association with obesity

Willy Theel[1 2], Vivianne de Jong[3 4], Stella Trompet[5 6], Diederick Grobbee[3 4], Elisabeth van Rossum[2], J. Wouter Jukema[5], Manuel Castro Cabezas[1 2 4]
[1]Franciscus Gasthuis, Internal Medicine, Rotterdam, Netherlands, *[2]Erasmus MC, Internal Medicine, Rotterdam, Netherlands*, *[3]Julius Center for Health Sciences and Primary Care, Utrecht, Netherlands*, *[4]Julius Clinical, Zeist, Netherlands*, *[5]Leiden University Medical Center (LUMC), Cardiology, Leiden, Netherlands*, *[6]Leiden University Medical Center (LUMC), Gerontology and Geriatrics, Leiden, Netherlands*
Email: w.theel@franciscus.nl

Background and aims: Metabolic dysfunction Associated Steatotic Fatty Liver Disease (MASLD) is a multifactorial condition in which body weight, lifestyle and genetic background are important determinants. Several genetic markers associated to MASLD have been published, but information on the prevalence in European cohorts is limited. Genome-wide association studies (GWAS) data have suggested that the PNPLA3 rare allele may be present in 23% of the European population and the GCKR rare allele in 60%. In the present study we evaluated the prevalence of these markers and of recently published risk alleles in an elderly European cohort. In addition, the relationship between these genetic markers for MASLD and obesity was analyzed.

Method: The PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) database was used for this study. Participants were divided into BMI subgroups : healthy (BMI <25 kg/m^2), overweight (BMI 25-29, 9 kg/m^2) and obese (BMI ≥30 kg/m^2). A complete GWAS was performed in the PROSPER/PHASE study using Illumina 660-Quad beadchips. 30 single nucleotide polymorphisms (SNPs) which have been associated with MASLD were evaluated using PLINK software.

Results: 5244 PROSPER participants from Caucasian descent in Scotland, Ireland and the Netherlands had available DNA samples. 1793 participants (34.2%) had a healthy BMI, 2378 (45.3%) had overweight and 1073 (20.5%) had obesity. The mean age (± SD) was 75.3 (± 3.4) years for the groups. Six risk alleles were the most informative in this cohort. The minor allele frequency (MAF) of these 6 SNP's was very similar between the 3 countries, albeit somewhat higher in Ireland except for the PNPLA3 allele which was the highest in the Netherlands (table 1). Five risk alleles showed a negative association with BMI : GCKR_rs1260326, GCKR_rs780094, C2ORF16_rs1919127, ZNF512_rs2068834 and PNPLA3_rs738409. The highest prevalence was found for GCKR_rs1260326 (0.41) whereas the PNPLA3_rs738409 had the lowest prevalence (0.19) in the cohort. The APOL3_rs1329665 showed a positive trend with BMI despite a low frequency.

Conclusion: The current study provides information on the prevalence of risk alleles for MASLD in an elderly population in 3 different European countries sharing the same Caucasian ethnicity. The data suggest a lower prevalence than previously reported. A negative relationship with BMI was found for most of these alleles. However, APOL3_rs132665 MAF was higher in patients with obesity in this elderly cohort. The role of the APOL3 gene in the context of obesity remains elusive, necessitating further investigation to ascertain its influence on lipid transport and/or binding within the pathophysiology of MASLD.

Figure: Table 1-MAF of the 6 SNPs per country

	Scotland	Ireland	Netherlands
GCKR_rs1260326	0.38	0.41	0.39
GCKR_rs780094	0.37	0.41	0.37
C2ORF16_rs1919127	0.25	0.28	0.25
ZNF512_rs2068834	0.26	0.29	0.27
PNPLA3_rs738409	0.19	0.19	0.22
APOL3_rs1329665	0.16	0.17	0.15

PO4-05-YI
Single-cell profiling of hepatic dendritic cells in metabolic dysfunction-associated steatotic liver disease

Camilla Klaimi[1], Pauline Jacquemain[1], Kevin Ory[1], Charlène Pourpe[1], Anne-Sophie Jenequin[1], Arnaud Carrier[2], Alexandre Pelletier[2], Fabien Delahaye[1], Amelie Bonnefond[2], David Dombrowicz[1], Bart Staels[1], Delphine Eberle[1], Joel Haas[1]
[1]Univ. Lille, Inserm, CHU Lille, Institut Pasteur Lille, U1011-EGID, F-59000 Lille, Lille France, *[2]Univ. Lille, Inserm, CHU Lille, Institut Pasteur Lille, U1283-UMR8199-EGID, F-59000 Lille, Lille France*
Email: camilla.klaimi@pasteur-lille.fr

Background and aims: Metabolic dysfunction-Associated Steatotic Liver Disease (MASLD) is the most common chronic liver disease worldwide, with its prevalence rising in concert with rates of obesity. MASLD is characterized primarily by hepatic triglyceride (TG) accumulation, called steatosis, which can progress to Metabolic dysfunction-Associated SteatoHepatitis (MASH), which combines steatosis with elements of hepatic necroinflammation and fibrosis. Our group previously demonstrated that altered hepatic conventional dendritic cell (cDC) populations are associated with MASH in humans and mice, with the ratio of cDC type 1 (cDC1) to cDC type 2 (cDC2) being decreased. Different animal models of cDC1 depletion displayed conflicting results with respect to protection from developing MASH. Here we aimed to comprehensively profile hepatic cDCs in a murine model of MASH and identify factors affecting their phenotype.

Method: Single cell RNAseq (scRNAseq) was performed on cDC sorted from livers from chow and NASH-diet fed mice. For in vitro studies, we used bone-marrow derived progenitors which were differentiated into cDC1- or cDC2-like cells using FLT3L+GMCSF or GMCSF alone, respectively (BMDC). On day 14 of the differentiation protocol, BMDC were cultured under various stimulations and collected after 24 hours for analysis of gene expression by RT-qPCR and the metabolic phenotype by Seahorse Extracellular Flux analyzer.

Results: Our scRNAseq results identified 9 cDC clusters in livers from chow and NASH-diet fed mice. When comparing the proportion of cells in each cluster, cluster 4 was found to be highly enriched in cells from NASH-diet fed mice (1.7% in Chow vs 10.7% in NASH). This cluster was highly enriched in markers of migratory dendritic cells, including the canonical migratory receptor *Ccr7.* Further investigation of marker genes in this cluster revealed a signature similar to mregDC, a DC phenotype closely associated with the tumor microenvironment. Gene expression studies in BMDC showed that certain mregDC markers are induced upon TLR stimulation and could be further induced by free fatty acid treatment.

Conclusion: Together, our results suggest changes in the metabolic environment during MASLD induce the mregDC transcriptional program. Further investigation is necessary to identify the precise signals driving mregDC induction and their role in MASLD progression.

PO4-07
MASLD and chronic viral hepatitis: baseline results from patients enrolled in the PITER HCV and HBV and HDV italian cohorts

Maria Giovanna Quaranta[1], Luigina Ferrigno[1], Maria Elena Tosti[1], Loreta Kondili[1 2], Piter Collaborating Group[3]
[1]Italian National Institute of Health, Roma, Italy, *[2]Università UniCamillus | Saint Camillus International University of Health Sciences, Roma, Italy*, *[3]PITER Collaborating group: names and affiliations of each member of this study group are listed in www.progettopiter.it*
Email: loreta.kondili@iss.it

Background and aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) encompasses patients who have hepatic steatosis and at least one of five cardiometabolic risk factors. The coexistence of other forms of liver disease as MASLD with viral hepatitis is also recognized. There are no accurate data neither on the prevalence of MASLD in HCV or HBV induced chronic liver disease nor on the liver disease progression due to MASLD in patients who achieved viral HCV elimination/or HBV disease control. We evaluated the prevalence and clinical characteristics of MASLD large HCV and HBV (HDV coinfected or not) cohorts.

Method: The data were retrieved from the Italian PITER cohorts of patients with chronic HCV (median age 63 years, IQR 52-71), HBV (median age 59 years, IQR 47-68) and HBV/HDV (median age 55 years, IQR 45-62), enrolled consecutively from more than 60 clinical centers distributed throughout Italy. Patients with HIV co-infection were excluded. Potential MASLD was defined taking into account the presence of steatosis and at least one of the metabolic dysfunctions included in the New Non-alcoholic fatty Liver Disease (NAFLD) Nomenclature. Adjusted odds ratios for potential confounding variables were calculated by multiple logistic regression analyses.

Results: The prevalence of MASLD was 21.2% (756/3571), 22.7% (2640/11619) and 12.6% (47/372) in the HBV, HCV and HBV/HDV cohorts, respectively ($p < 0.001$). In the HCV cohort, the prevalence of MASLD remained 23.0% also excluding 1077 patients with genotype 3. In patients with liver cirrhosis, the prevalence of MASLD in the HBV, HCV and HBV/HDV cohorts was 24.7% (207/838), 20.0% (958/4787) and 12.6% (33/261), respectively ($p < 0.001$). Similarly, in patients with F0-F3 fibrosis stage the prevalence of MASDL was significantly higher in HCV chronic infected patients compared with HBV and HBV/HDV chronic infected patients, specifically: 23.7% (1148/4854) in HCV vs 20.1% (549/2733) in HBV and 12.6% (14/111) in HBV/HDV cohorts ($p < 0.001$). No difference was observed in the prevalence of steatosis between HCV and HBV (HDV coinfected or not) patients' cohort (27.4% vs 26.9%; $p = 0.473$). The prevalence of cardiovascular diseases, diabetes and cirrhosis were significantly higher ($p < 0.001$) in HCV versus HBV patients' cohort (37.9% vs 25.1%; 14.1% vs 9.6%; 49.7% vs 27.9%, respectively) whereas alcohol use and BMI>25 were more frequently present in HBV versus HCV patients' cohort (22.0% vs 15.6%; 53.7% vs 50.7%, respectively, $p < 0.005$). These results were confirmed after adjusting for potential confounders. Further analysis showed that MASLD was independently associated with older age (OR 1.02 CI95%1.01 -1.02), male gender (OR 1.39 CI95% 1.28-1.51), HCV infection (OR 1.10 CI95% 1.00 - 1.21) and a fibrosis stage different from cirrhosis (OR 1.30 CI95% 1.19-1.42).

Conclusion: Different prevalence of MASLD was observed in patients with HCV, HBV and HBV/HDV chronic infection and in those with different stages of liver disease due to viral hepatitis. Prospective evaluations are necessary to evaluate if MASLD is a cofactor or bystander on HCV, HBV and HBV/HDV disease progression.

PO4-08
Comparison of the efficacy of MR spectroscopic and MR imaging methods for assessing liver fat changes in obese patients undergoing dietary intervention and GLP-1 agonist treatment

Petr Kordač[1], Dita Pajuelo[2], Milan Hájek[2], Martin Burian[2], Petr Šedivý[2], Monika Dezortová[2], Jan Kovář[1]
[1]Institute for clinical and experimental medicine, CEM, Praha 4, Czech Republic, *[2]Institute for clinical and experimental medicine, ZRIR, Praha 4, Czech Republic*
Email: kodp@ikem.cz

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in adults. One of the early signs of NAFLD is hepatic steatosis, which is characterised by an increase in hepatic fat content (HFC) that can be diagnosed using biopsy or imaging methods. In this study, we compared four different MR methods for assessing HFC in obese subjects undergoing dietary intervention with or without the agonist of GLP-1.

Method: Sixteen obese non-diabetic men (age: 47.0 ± 12.4 years, BMI: 36.2 ± 3.8 kg/m2) with HFC >4% underwent a 16-week dietary intervention (nutritional counselling) and 16-week treatment with GLP-1 agonist (semaglutide 0.25-1 mg/week; Ozempic®). The order of both interventions was randomised. All participants underwent seven MR examinations-three before the study (each at least two weeks apart) and two at week 14 and 16 of each intervention period. MR examinations were performed using 3T MR system VIDA (Siemens, Germany). The examination protocol included standard Siemens LiverLab protocol containing proton density fat fraction measurement using VIBE e- and q-Dixon sequences and automatic spectroscopy sequence HISTO. Moreover, our laboratory liver spectroscopic protocol was applied (STEAM sequence). STEAM and HISTO volume of interest (40×30×25 mm) and VIBE roi were placed in the liver segment V/VIII. HFC from whole liver volume (VIBE all liver) was measured by q-Dixon images using liver mask segmented from e-Dixon images. The results of fat fraction were recalculated to HFC using Longo correction.

Results: Dietary intervention did not significantly affect neither body weight nor HFC quantified by all four methods whereas semaglutide treatment led to significant decrease in both body weight and HFC. The results obtained by HISTO and VIBE all liver (12.4 ± 8.5 and 12.9 ± 7.4%, respectively) were approximately 10% higher than those obtained by STEAM and VIBE roi (11.1 ± 7.5 and 11.4 ± 7.9%, respectively). However, results obtained using all the methods were highly correlated (Table 1). Although HISTO is based on automatic T2 relaxation time measurement only from TEs = 12-72 ms, compared to manual STEAM measurement with TEs = 20-270 ms, HISTO is faster and more user-friendly than the STEAM method because spectra from STEAM have to be manually evaluated. On the other hand, slightly higher HFC assessed by HISTO protocol compared to our STEAM protocol can be attributed to shorter T2 relaxation times obtained by HISTO. HFC calculated from the whole liver volume revealed the weakest correlation with the other methods especially because of occasional bad liver segmentation and counting bile ducts. All methods showed statistically similar HFC in both examined groups.

Conclusion: All of the listed MR methods are suitable for monitoring of HFC changes during therapeutic and lifestyle interventions. Supported by MH CR, grant nr. NU20-01-00121.

Figure:*Pearson correlation coefficient (R2) between different MRS and MRI methods used for HFC measurement*

	STEAM [R2]	HISTO [R2]	VIBE roi [R2]	VIBE all l. [R2]
STEAM [R2]		0.99	0.94	0.93
HISTO [R2]	0.99		0.94	0.92
VIBE roi [R2]	0.94	0.94		0.92
VIBE all l. [R2]	0.93	0.92	0.92	

PO4-09-YI
Gender differences in the development of steatotic liver disease and fibrosis: implications for diagnosis and management

Florian Koutny[1], Bernhard Wernly[2], Elmar Aigner[3], Christian Datz[2], Sophie Gensluckner[3], Andreas Maieron[1], Stefano Novati[4], Bernhard Iglseder[5], Patrick Langthaler[6], Vanessa Frey[6], Barnhard Paulweber[3], Eugen Trinka[6]
[1]University Hospital St. Pölten, Gastroenterology and Hepatology, Sankt Pölten, Austria, *[2]General Hospital Oberndorf, Department of Internal Medicine, Teaching Hospital of the Paracelsus Medical University, Salzburg, Austria*, *[3]University Hospital Salzburg, First Department of Medicine, Salzburg, Austria*, *[4]Bolzano Regional Hospital, Gastroenterology Department, Bolzano, Italy*, *[5]Christian-Doppler-Klinik, Department of Geriatric Medicine, Salzburg, Austria*, *[6]Department of Neurology, Christian Doppler University Hospital, Paracelsus Medical University and Centre for Cognitive Neuroscience, Member of the European Reference Network EpiCARE*
Email: florian.koutny@stpoelten.lknoe.at

Background and aims: Steatotic liver disease (SLD) is a serious health concern with an increasing prevalence worldwide. Only a few small studies have highlighted the role of sex in its development and progression. Therefore, in this study, we want to investigate the association between sex and the risk of SLD and liver fibrosis, while controlling for potential confounding factors

Method: Our study included 8, 315 participants from the Paracelsus 10, 000 study. We used multivariable logistic regression models and multivariable linear regression to assess the association between SLD and sex. The primary end points were increased fatty liver index (FLI) score ≥60 and liver fibrosis as indicated by an elevated fibrosis-4 index (FIB-4) score ≥1.3 as surrogate markers. Participants with viral hepatitis or excessive alcohol consumption (>40g in females and >60g in males) were excluded from this study.

Results: Males had higher levels of various health indicators and were more likely to have dyslipidemia, diabetes 2, metabolic syndrome, and FLI compared to females. Among the female population, the odds of having a FLI >60 were significantly lower than males, with a baseline odds ratio (OR) of 0.28 (95% CI: 0.26-0.32, p < 0.01). This association remained true after adjusting for age and MetS (model 2) and Score 2 (model 3) with an OR 0.24 (95% CI: 0.22-0.27, p = 0.01) and OR of 0.44 (95% CI: 0.40-0.49, p = 0.01) respectively. Women aged <55 years had lower odds of SLD than females <55 years (OR = 0.24, 95% CI: 0.21-0.28, p = 0.01). Similarly, after adjusting for MetS females had lower odds of SLD, with ORs of 0.22 (95% CI: 0.19-0.26, p = 0.01) and 0.26 (95% CI: 0.23-0.31, p = 0.01) for age <55 and >55 years, respectively. Females also had lower odds of fibrosis, even after adjusting for age and MetS, but there was no significant association after accounting for Score 2.

Conclusion: The study's findings suggest that sex plays a significant role in the development of SLD and liver fibrosis, with females exhibiting significantly lower odds than males. Postmenopausal women are also found to have a higher prevalence of metabolic syndrome compared to premenopausal women. These results highlight the need to consider sex as a crucial factor in the diagnosis and management of SLD, and to implement sex-specific screening and prevention strategies. Our study provides valuable insights that could impact clinical practice and public health policies, underscoring the importance of understanding sex differences in SLD and liver fibrosis.

PO4-10
Treatment with semaglutide affects favorably hepatic fat content but has no effect on response of liver fat to glucose and/or fructose administration

Jan Kovar[1], Tereza Dusilova[1], Petra Svobodova Stastna[1], Petr Sedivy[1], Dita Pajuelo[1], Monika Dezortova[1], Ivana Lankova[1], Lenka Thieme[1], Milan Hajek[1]
[1]*Institute for Clinical and Experimental Medicine, Prague 4, Czech Republic*
Email: jan.kovar@ikem.cz

Background and aims: Fat accumulation in the liver is a key step in the development of NAFLD. Using magnetic resonance (MR) spectroscopy, we demonstrated that nutrient-induced changes in hepatic fat content (HFC) can be detected even within a few hours. Repeated administration of glucose (3 x 50 g) resulted in a 15% reduction in liver fat content in healthy male volunteers 6 hours later. No such effect was observed when the same amount of fructose was administered (Dusilova et al: Am J clin Nutr 2019, 109: 1519). However, such an effect of repeated glucose administration on HFC was not observed in subjects with steatosis. In this study, we attempted to determine whether treatment of obese subjects with GLP-1 receptor agonist semaglutide (Ozempic®) results in a reduction in HFC and whether it affects the acute HFC response to repeated loads of glucose and fructose.

Method: Sixteen obese non-diabetic men (age: 47.0 ± 12.4 years, BMI 36.2 ± 3.8 kg/m^2) underwent a 16-week dietary intervention and 16-week treatment with semaglutide (0.25-1 mg/week) in a cross-over design without washout period. The order of interventions was randomized. All the subjects underwent seven 8-hour examinations, three before the study at baseline and two at the end of each dietary intervention. In each of these examinations, the effect of repeated administration of glucose and/or fructose (3 x 50 g at 2-hour intervals) on HFC was measured before first sugar administration and 6 hours later. The amount of subcutaneous and visceral fat were also determined using MR. The concentrations of glucose, triglyceride, non-esterified fatty acids, 3-hydroxybutyrate, insulin and glucagon were monitored during all examinations.

Results: The 16-week treatment intervention resulted in a 7.0 ± 4.3% reduction of weight of the subjects from 120.5 ± 12.7 kg to 112.0 ± 12.3 kg, p <0.001, and in a 35.3 ± 24.2% reduction of HFC from 14.1 ± 8.4 % to 8.4 ± 5.1 %, p <0.01. The treatment also favorably affected insulin sensitivity, most importantly the response of glucose to repeated glucose load. No acute HFC change was observed after both glucose and fructose administration at baseline as well as after both dietary intervention and semaglutide treatment. Dietary intervention resulted also in decrease of both weight and HFC, but the effect was less pronounced. Interestingly, the change in HFC after dietary intervention correlated with change in BMI and body fat, whereas no such correlation was found after semaglutide treatment (Table 1).

Conclusion: It can be concluded that treatment with semaglutide favorably affects both body weight and HFC, but does not affect the HFC response to repeated loads of glucose and fructose.
Supported by Ministry of Health of the Czech Republic, grant nr. NU20-01-00121.
Figure:

Table 1. The correlation coefficients between change of hepatic fat content (Δ HFC) and change of selected anthropometric parameters after 16-week dietary intervention and 16-week semaglutide treatment

	Δ body weight [kg]	Δ BMI [kg.m^{-2}]	Δ tot. body fat [kg]	Δ subc. fat [cm^2]	Δ visceral fat [cm^2]
dietary treatment	0.888 ***	0.933 ***	0.882 ***	0.763 ***	0.781 ***
semaglutide treatment	0.284 ns	0.338 ns	0.021 ns	0.109 ns	0.720 **

, *... p <0.01, p <0.001, ns ... not significant

PO4-13-YI
Low number needed to screen for advanced MASLD fibrosis in internal medicine and general practice

Koen van Son[1 1], Stan Driessen[2], Joost Drenth[1], Maarten Tushuizen[3], Onno Holleboom[2]
[1]Radboudumc, Gastroenterology and Hepatology, Nijmegen, Netherlands, *[2]Amsterdam UMC, Vascular medicine, Amsterdam, Netherlands*, *[3]LUMC, Gastroenterology and Hepatology, Leiden, Netherlands*
Email: k.c.vanson@amsterdamumc.nl

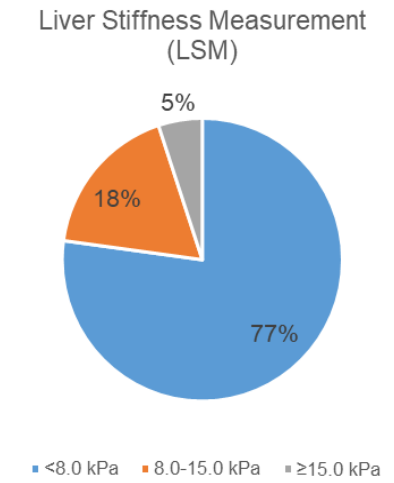
Background and aims: In the implementation of diagnostic algorithms for MASLD, optimal incorporation of non-invasive liver fibrosis tests (NITs) across the lines of care is essential, but comparative studies on this matter are limited. To this end, we initiated the NLA2 care path study, the first MASLD care path in The Netherlands, aiming to determine the optimal diagnostic algorithm to identify patients with MASLD and advanced fibrosis across primary, secondary and tertiary care.

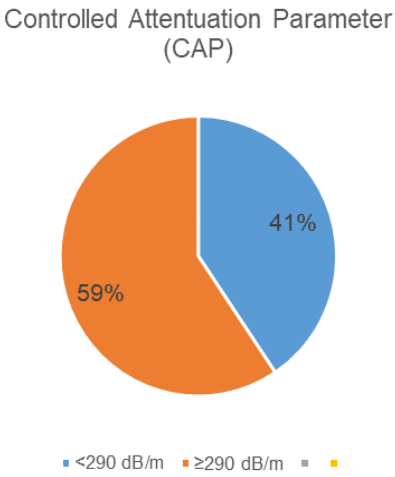
Method: General physicians (GPs) and internists from two regional and one academic hospital referred patients at risk for MASLD: presence of type 2 diabetes mellitus (T2DM), adiposity, metabolic syndrome (MetS), elevated liver enzymes and/or steatosis on ultrasound. Exclusion criteria were previously diagnosed cirrhosis or other chronic liver diseases. During a single study visit, hepatic steatosis and fibrosis were assessed using FIB4 and vibration controlled transient elastography (VCTE, FibroScan®). Patients with elevated FIB4 (≥3.25) and/or Liver Stiffness Measurement (LSM) (≥8.0 kPa) were considered at high likelihood of advanced fibrosis and subsequently referred to the MASLD clinic. Plasma was stored for future determination of other NITs, including Enhanced Liver Fibrosis (ELF)-test and new fibrosis signatures.

Results: From the first 207 participants enrolled in the study, 179 came from a hospital setting and 32 from primary care. 12 participants were excluded due to self-reported excessive alcohol intake. 198 participants were included in the analysis. The mean age was 57.7 years (SD 12.7). 57.1% of participants was male and 56.6% had a BMI ≥30 kg/m^2. T2DM was present in 52.0%, with a median HbA1c of 58.0 [49.5; 65.0] mmol/mmol and 76.4% met the criteria for MetS. 59.4% had a CAP ≥290 dB/m^2 and 22.8% had LSM ≥8.0 kPa, yielding a number needed to screen (NNS) of 4.4 to detect one case of advanced fibrosis. The NNS in GP practices and hospitals were 7.2 and 4.1, respectively. 5.1% had LSM ≥15.0 kPa, suggesting cirrhosis, yielding a NNS of 19.6 to detect one case of cirrhosis. 65.9% had FIB4 <1.30, 33.0% had FIB4 between 1.30 and 3.25, and 1.1% had FIB4 ≥3.25. Of note, LSM and FIB4 did not correlate (Spearman's rho: 0.12 ($p = 0.10$)). FIB4 often was false-negative or false-positive: 57.5% of participants with LSM ≥8.0 kPa had FIB4 <1.30 and 31.9% with LSM <8.0 kPa had FIB4 ≥1.30. Sensitivity and negative predictive value (NPV) for the lower cut-off of 1.30 were 0.43 and 0.81, respectively.

Conclusion: In these first data from our NLA2 care path study 22.8% of participants had LSM ≥8.0 kPa and 5.1% had LSM ≥15.0 kPa. Thus, we found a remarkably low NNS of 5 to detect one case of advanced fibrosis and 20 for cirrhosis in our cohort of patients with clinical risk of MASLD across three lines of care. Of note, FIB4 and LSM did not correlate and FIB4 was often false-negative or false-positive, which may point to limited diagnostic value of FIB4 in lower prevalence populations and the need to determine the optimal NIT sequence in our ongoing study.

Figure:





High prevalence of advanced MASLD fibrosis in internal medicine and general practice**.**

PO4-15-YI
Multiparametric liver ultrasound for non-invasive quantification of steatosis, fibrosis, ballooning and inflammation in metabolic dysfunction-associated steatotic liver disease

Antonio Liguori[1 2], Maria Elena Ainora[1 2], Luca Di Gialleonardo[1 2], Maria Cristina Giustiniani[2 3], Fabio Maria Vecchio[2 3], Antonio Gasbarrini[1 2], antonio grieco[1 2], Luca Miele[1 2], Maria Assunta Zocco[1 2]
[1]Department of Medical and Abdominal Surgery and Endocrine-Metabolic Sciences, Fondazione Policlinico Universitario A Gemelli IRCCS, Rome, Italy, *[2]Università Cattolica del Sacro Cuore*, *[3]Department of Women, children and public health sciences, Fondazione Policlinico Universitario A Gemelli IRCCS, Rome, Italy*
Email: luca.miele@policlinicogemelli.it

Background and aims: Metabolic-dysfunction Associated Steatotic Liver Disease (MASLD) is the most common chronic liver disease worldwide but identifying patients with Metabolic-dysfunction Associated steatohepatitis (MASH) and advanced fibrosis remains a challenge. In last few years many ultrasound (US)-based non-invasive diagnostic tools have been developed. They showed high reliability to assess liver fibrosis and liver steatosis but low accuracy in measure necro-inflammatory grade. Viscosity of liver tissue, measured through shear wave spectroscopy, is a new promising US tool for grading of the necro-inflammatory activity in liver diseases. The aim of this study was to assess the potential role of liver viscosity for diagnosis of MASH and for stratification of lobular inflammation and ballooning.

Method: We conducted a monocentric cross-sectional study collecting data from consecutive patients who underwent liver biopsy for suspected MASH between January 2019 and December 2021 at Fondazione Policlinico A. Gemelli in Rome. Two-dimensional shear wave elastography (2D-SWE), Sound Speed Plane-wave US (SSp.PLUS) and Viscosity Plane-wave US (Vi.PLUS) were measured using Aixplorer MACH 30 system immediately before liver biopsy, together with transient elastography (TE) and controlled attenuation parameter (CAP) from FibroScan. MASH was histologically defined as NAS□4 with at least 1 point for steatosis, ballooning and lobular Inflammation. A multivariate logistic regression analysis was carried out to build a model for prediction of MASH based on multiparametric US parameters. Bootstrap method was used for internal validation. Diagnostic performance was assessed by using the area under the receiver operating characteristic curve (AUROC). Calibration and decision curve analysis was carried out for quality assessment of the model.

Results: 120 patients were prospectively enrolled (mean age 49, 56% were male). Prevalence of diabetes, obesity and arterial hypertension was 29%, 60% and 45%, respectively. According to histopathological evaluation, prevalence of MASH and advanced fibrosis was 67% and 28%, respectively. CAP and SSp.PLUS increases progressively with steatosis grade ($p < 0.01$). Viscosity increases progressively as the degree of lobular inflammation and ballooning increases ($p < 0, 01$). The diagnostic performance of Vi.PLUS for the presence of both ballooning grade≥1 and lobular inflammation≥1 was good with an AUROC of 0.72. At multivariate logistic regression analysis three independent predictors of MASH were selected: Vi.PLUS, AST and SSp.PLUS. A score based on those variables was highly accurate for the diagnosis of MASH (AUROC 0.75) and a showed an excellent goodness of fit of predicted probability of NASH. Both TE and 2D-SWE showed good performance for the diagnosis of advanced fibrosis (AUROC 0.93 and 0.90, respectively).

Conclusion: Viscosity is a promising tool for non-invasive assessment of lobular inflammation and ballooning in MASLD. A new score based on multiparametric US parameters and AST has a good diagnostic performance of MASH and can guide the decision to refer the patient for liver biopsy.

PO4-16-YI
Non-invasive assessment of cell-free circulating mtDNA and copy number open an intriguing spyhole for the diagnosis of NAFLD-HCC in genetically predisposed individuals

Miriam Longo[1], Erika Paolini[1 2], Marica Meroni[1], Michela Ripolone[1], Laura Napoli[1], Marco Maggioni[1], Maurizio Moggio[1], Anna Ludovica Fracanzani[1 2], Paola Dongiovanni[1]
[1]Policlinico of Milan, Milano, Italy, *[2]University of Milan, Milano, Italy*
Email: paola.dongiovanni@policlinico.mi.it

Background and aims: Mitochondrial (mt) dysfunction is a hallmark of progressive NAFLD, and it may be exacerbated by the cumulative weight of PNPLA3/MBOAT7/TM6SF2 loss-of-function, the major genetic predictors of the disease. However, the impact of these genes on mt-dynamics, in terms of mass, morphology and turnover, remains an uncharted filed and requires to be investigated. MtDNA copy number (mtDNA-CN) and cell-free circulating mtDNA (ccf-mtDNA), which reflect mt-mass and mt-dysfunction, respectively, are gaining attention for NAFLD non-invasive assessment. Therefore, we aim to assess the genetic contribution on mt-dynamics, mtDNA-CN and ccf-mtDNA in 1) primary mouse hepatocytes (PMHs) silenced for PNPLA3/MBOAT7/TM6SF2 genes; 2) Discovery (n = 28) and Validation (n = 824) cohorts, including biopsied NAFLD patients, stratified according to number of risk variants (NRV = 3).

Method: Mt-morphology was assessed by transmission electron microscopy (TEM). mtDNA-CN and ccf-mtDNA were measured in PBMCs and serum samples, respectively, of both Discovery and Validation cohorts. mtDNA-CN and mt-dynamics were evaluated in liver biopsies.

Results: PMHs challenged with fat overload or PNPLA3/TM6SF2/MBOAT7 co-silencing lowered mt-fusion paralleled by higher mt-fission and ccf-mtDNA release, suggesting that lipid accumulation and genetics may independently unbalance mt-dynamics. In the Discovery cohort, NRV = 3 patients showed the highest mtDNA-CN compared to those with 1-2 or no variants. At TEM, NRV = 3 carriers increased mt-mass and presented an elevated pattern of mt-morphological alterations (swollen shapes, double membranes rupture). Moreover, hepatic PGC1α expression, the master regulator of mt-biogenesis, was higher in NRV = 3 patients, supporting the increased mt-mass observed by TEM. In the Validation cohort, mtDNA-CN associated with the NAFLD histological spectrum and NRV = 3 at multivariate analyses, supporting that both NAFLD severity and genetics may modulate mt-dynamics. In liver biopsies, mtDNA-CN was higher in NRV = 3 patients together with reduction of mt-fusion and activation of mt-fission, resembling what observed in hepatocytes. Ccf-mtDNA was augmented in NRV = 3 patients with low-moderate/severe NAFLD, thereby sustaining that this effect was amenable to the three at-risk polymorphisms. By stratifying the Validation cohort according to the clinical phenotype, we found that circulating mtDNA-CN progressively increased from steatosis to HCC and ROC curves showed that it significantly discriminated HCC cases ($p < 0.0001$; AUC: 0.86), resulting a good candidate for NAFLD monitoring. Serum ccf-mtDNA increased in HCC patients and it foretold HCC onset ($p < 0.0001$; AUC: 0.91).

Conclusion: mtDNA-CN and ccf-mtDNA may have pathological and predictive significance in NAFLD patients at high-risk, especially in those genetically predisposed

PO4-17
Patients with autoimmune hepatitis and metabolic dysfunction-associated steatotic liver disease do not exhibit worse liver outcomes

Ellina Lytvyak[1], Aldo J Montano-Loza[1]
[1]*University of ALberta*
Email: lytvyak@ualberta.ca

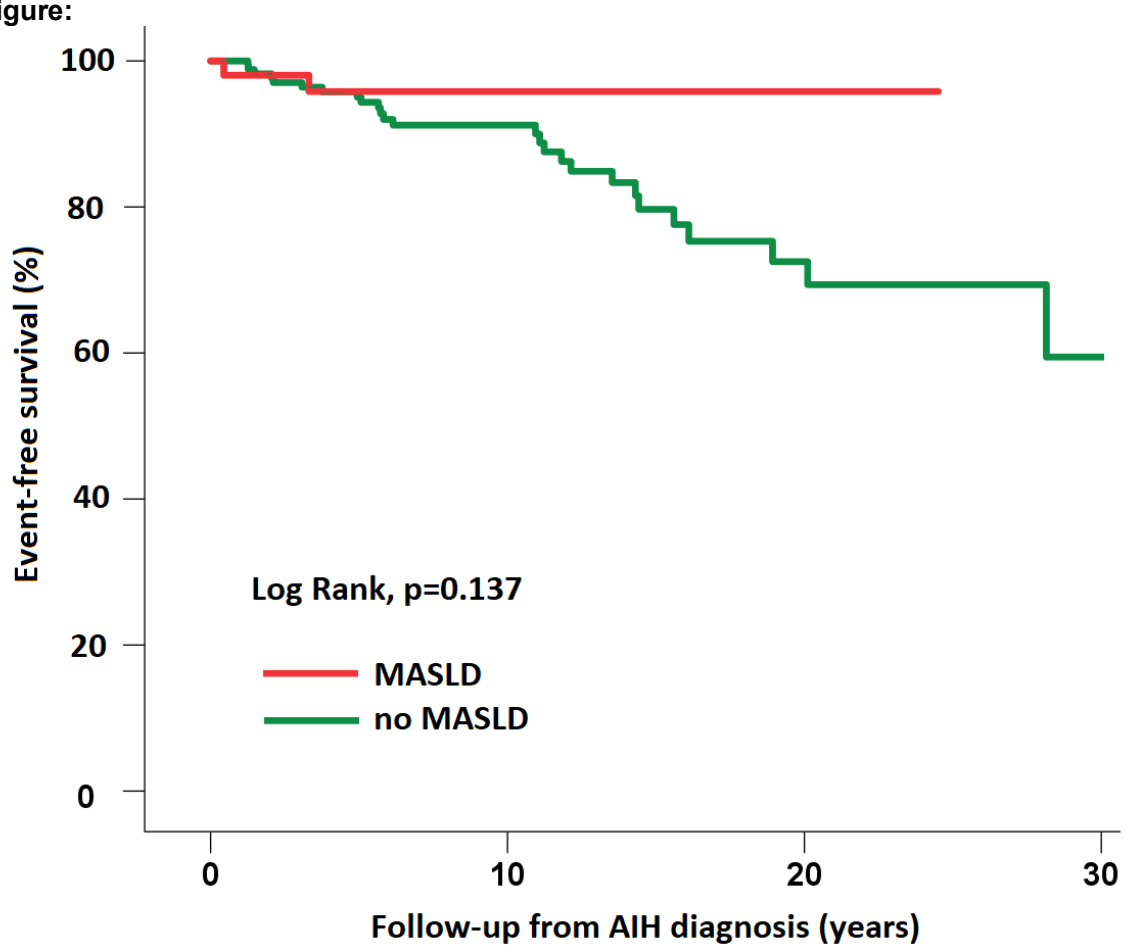
Background and aims: Due to a constantly rising prevalence of steatotic liver disease (SLD), excess weight and metabolic syndrome, data on clinical significance of hepatic steatosis and cardiometabolic criteria and their impact on adverse liver outcomes in patients with autoimmune hepatitis (AIH) are currently being explored. Our aim was to establish the prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) and determine its association between and adverse liver outcomes and quantify the magnitude of this association in patients with AIH.

Method: We conducted an observational, retrospective cohort, single-centre study of AIH patients followed at the Liver Unit, Division of Gastroenterology, University of Alberta, and diagnosed between 1971 and 2022. SLD was diagnosed by the vibration-controlled transient elastography (VCTE) with a controlled attenuation parameter (CAP)≥288 dB/min signifying fatty infiltration. Only VCTE measures meeting quality criteria with at least 10 validated measurements and an interquartile range ≤30% of the median liver stiffness measurement value were included. Only patients with data available for evaluation of all five sets of cardiometabolic criteria (CMC: body mass index, glycemic, blood pressure, triglycerides and HDL profiles) were eligible. Diagnosis of MASLD was established based on the presence of SLD and at least one CMC. Chi-square test was used to compare proportions, Mann-Whitney test-to compare medians. Survival probabilities were calculated based on the Kaplan-Meier methodology and the significance of differences by the log-rank (Mantel-Cox) test. The Cox proportional hazards regression model was utilized with calculation of hazard ratios (HR) with 95% confidence intervals (95%CI).

Results: We analyzed data of 228 AIH patients with a Simplified Autoimmune Hepatitis Score ≥6, 71.9% females, 85.3% Caucasians, median age at diagnosis 46.2 [range 6-96] years old, and median disease duration of 8.9 [range 0.1-44.4] years (2, 388 person-years). SLD prevalence was 22.8% (n = 52) without any substantial difference between sexes (22.6% (n = 37) among females vs. 23.4% (n = 15) among males; p = 0.887). All patients diagnosed with SLD had at least 1 of the CMC, with most common being overweight (92.3%, n = 48); thus, all of them met criteria of MASLD. Cirrhosis at diagnosis tended to be more common among AIH patients with MASLD compared to those without (25.0% vs. 14.8%; p = 0.085). MASLD was not associated with either development of cirrhosis (HR 0.36, 95%CI 0.09-1.53; p = 0.167), development of decompensation (HR 0.23, 95%CI 0.03-1.74; p = 0.156), or liver transplantation or death (HR 0.34, 95%CI 0.08-1.42; p = 0.137) (Fig. 1). Event-free survival tended to be longer among non-MASLD AIH patients (median 9.1 [0.4-38.6] vs. 6.9 [0.1-24.5] years, p = 0.104), the same tendency was observed for overall survival (median 9.2 [0.4-44.4] compared to 6.9 [0.1-24.5] years, p = 0.071).

Conclusion: In our large Western Canadian cohort, MASLD was present in over 20% of AIH patients. Cirrhosis at diagnosis tended to be more frequent among AIH patients with MASLD compared to those without. We did not identify any significant difference in adverse liver outcomes between AIH patients with and without MASLD. Further multi-centre studies with larger sample size are warranted to explore potential impact of MASLD on adverse liver outcomes among AIH patients.

Figure:


100
80
60
40
20
0
Event-free survival (%)
Log Rank, p=0.137
MASLD
no MASLD
0
10
20
30
Follow-up from AIH diagnosis (years)

PO4-18
Metabolic-associated steatotic liver disease and insulin-related mechanisms: insights from a cluster analysis of the PREVADIAB2 study

Maria-Paula Macedo[1 2], Maria Joao Meneses[1 3], Ana Pina[1], Barbara Patrício[4], Fabrizia Carli[4], Rogerio Ribeiro[2], Joao-Filipe Raposo[1 2], Amalia Gastaldelli[4]
[1]NOVA Medical School, NOVA University of Lisbon, Lisbon, Portugal, *[2]APDP-Diabetes Portugal, Education and Research Center, Lisbon, Portugal*, *[3]DECSIS II Iberia, Lda, Évora, Portugal*, *[4]National Research Council (CNR), Institute of Clinical Physiology (IFC), Pisa, Italy*
Email: paula.macedo@nms.unl.pt

Background and aims: Metabolic-associated steatotic liver disease (MASLD) is highly related with insulin-related mechanisms. Indeed, as insulin is lipogenic, it plays a key role in modulating lipid metabolism and homeostasis. Therefore, the aim of this study is to investigate the MASLD profiles of individuals within different clusters based on insulin-mediated mechanisms, and to examine potential sex differences in these profiles.

Method: To assess the interplay between MASLD and insulin-related mechanisms, we performed hierarchical clustering analysis of PREVADIAB2 cohort (n = 953 subjects that had no diabetes 5 years before) based on fasting insulin secretion (fISR), clearance (fIC), and resistance (HOMA-IR) and insulinogenic index (IGI) during oral glucose tolerance test. The resulting clusters were profiled for steatotic liver disease, and the lipidome of 273 females and 215 males was assessed by LC/MS-QTOF.

Results: Among the subjects of PREVADIAB2 here reported, 33% had fatty liver index (FLI) >60 and 32% had a FLI <30. Four clusters were identified and named according to their metabolic hallmarks: Liver Sensitive (LS); Pancreas Glucose Sensitive (PGS); Insulin Deficient (ID); and Insulin Resistant (IR). The latter present the highest hepatic insulin resistance, with the highest value for women. Concomitantly, IR was also the cluster presenting higher NAFLD-FLS, with women presenting higher values than men ($p < 0.01$), followed by the PGS cluster. Furthermore, only 35% of subjects in IR were excluded from having fibrosis using fibrotic NASH index (FNI). On the contrary, LS cluster had the lowest hepatic steatosis (estimated by FLI and NAFLD-FLS) and the most favorable lipid profile, whereas the other clusters showed lipid alterations associated to insulin dysmetabolism. Although the pattern of insulin related parameters' were similar between sexes among clusters, the hepatic and lipidomic phenotypic profiles differed. Surprisingly, women had lower hepatic enzymes (AST, ALT and GGT) and FNI, but higher NAFLD-FLS than men, especially in the IR cluster. Furthermore, women presented lower lysophosphatidylcholine (LPC) and long chain ceramides (CER 26:1) and higher sphingomyelins (SM) compared to men. In the IR cluster, TG50:0 and TG52:0 was overall increase, but women also had a significant increase in PC32:1 and PC34:1.

Conclusion: Our data shows that insulin is highly connected to lipid profile and SLD. Furthermore, our results highlight the importance of considering sex differences in hepatic, metabolic, and lipidomic profiles suggesting that sex-specific cut-offs might be used when studying conditions such as MASLD.
Acknowledgements: EU Horizon 2020, under Marie Sklodowska-Curie Grant Actions, Agreements N. 722619 and 734719 and EU Horizon Europe, agreement n. 101080329 (PAS GRAS).

PO5-01
Association of liver fibrosis with emerging extrahepatic cancer in fatty liver patients with the PNPLA3 I148M GG genotype: an 8-year cohort study

M Chang[1]
[1]*Taiwan, Department of Gastroenterology and Hepatology, Chang Gung Memorial Hospital, Linkou, Taoyuan, Taiwan*
Email: mlchang8210@gmail.com

Background and aims: Impacts of patatin-like phospholipase domain-containing protein 3 (PNPLA3) I148M-rs738409, methylenetetrahydrofolate reductase (MTHFR) Ala222Val-rs1801133 and aldehyde dehydrogenase 2 (ALDH2) Glu504Lys-rs671 on the outcomes of Taiwanese patients with fatty liver have remained elusive.

Method: An 8-year prospective cohort study of patients with (n = 546) and without (n = 580) fatty liver (controls) was conducted in a Taiwanese tertiary care center.

Results: The 546 fatty liver patients comprised 306 (56.0%) males and 240 (44.0%) female, with mean ages of 53.3 and 56.4 years, respectively. Compared with controls, fatty liver patients had an increased frequency of the PNPLA3 I148M-rs738409 GG genotype (25.5 vs. 5.9%, p = 0.001). Of fatty liver patients, 236 (43.1%) suffered cardiovascular events; 52 (9.5%) suffered extrahepatic cancers; 13 (2.38%) suffered hepatic events, including hepatocellular carcinoma (n = 3, 0.5%) and liver cirrhosis (n = 5, 0.9%); and none died. Fibrosis-4 (FIB-4) scores were associated with extrahepatic cancer (HR: 1.325; 95% CI: 1.038~1.691) and with cirrhosis development (HR: 1.532; 95% CI: 1.055~2.224). PNPLA3 I148M-rs738409 G allele (β = 0.158, 95% CI: 0.054~0.325) was associated with FIB-4 score. Stratified analyses showed, impact of the FIB-4 score on extrahepatic cancer development was evident only in fatty liver patients with PNPLA3 I148M-rs738409 GG genotype (HR: 1.543; 95% CI: 1.195~1.993) and not in patients with GC or CC genotype. ALDH2 Glu504Lys-rs671 G allele has a dose effect on alcoholism. MTHFR and ALDH2 genotypes were not significantly associated with fatty liver patients' outcomes.

Conclusion: Special vigilance should be exercised for emerging extrahepatic cancer in fatty liver patients with PNPLA3 I148M-rs738409 GG genotype and high FIB-4 scores.

PO5-02-YI
The correlation between the LDL level and the development of NAFLD

Mohamed Shafi Mahboob Ali[1]
[1]*Advanced medical and Dental Institute (AMDI), General Surgery, Penang, Malaysia*
Email: mshafix_7@yahoo.co.uk

Background and aims: NAFLD is a fast growing liver pathology in the world that is associated with many factors such as obesity, body cholesterol level, Diabetes Mellitus, hypertension, genetics and others. The end result of NAFLD is liver cirrhosis and hepatocellular carcinoma which usually takes about five to ten years to develop. Till this day, we are still struggling to combat NAFLD progression as there are no magic bullets available. The current guideline suggests patients diagnosed with NAFLD to treat the underlying comorbidities and adopt a healthy lifestyle. The aim of this study is to examine the correlation between the LDL levels with the development of NAFLD in our center.

Method: This is a retrospective study in which patients that were diagnosed with NAFLD were demographically studied and documented in the institute's online data base. A total of 1000 patients were included. Variables such as the age, sex, ethnicity, BMI, comorbidities, smoking status, liver function test and fasting serum lipid were recorded. Data from year 2015 till 2020 were analyzed using the software empower stats version 5.0. All statistically significant P value of less than 0.05 were taken into account. Multivariate non-parametric analysis of the mean data were studied using MANOVA test with the confident interval (CI) of 95%. In order to study the LDL correlation with the NAFLD, receiver operating characteristic (ROC) curves were plotted. The area under the curve (AUC) were calculated and DeLong test was used to determine the significance of the data. NAFLD was defined using the liver ultrasound criteria such as liver brightness, vascular blurring, deep attenuation, and hepatorenal echo contrast. The scans were performed by the trained ultra-sonographer to reduce the operator bias.

Results: The level of LDL in both men and women were statistically significant in the development of NAFLD.ROC analysis for both sexes were performed and AUC for LDL was significantly higher in the NAFLD patients.

Conclusion: The level of LDL is significantly higher in NAFLD patients in both sexes and can be utilized as an early biomarker for the development of NAFLD.

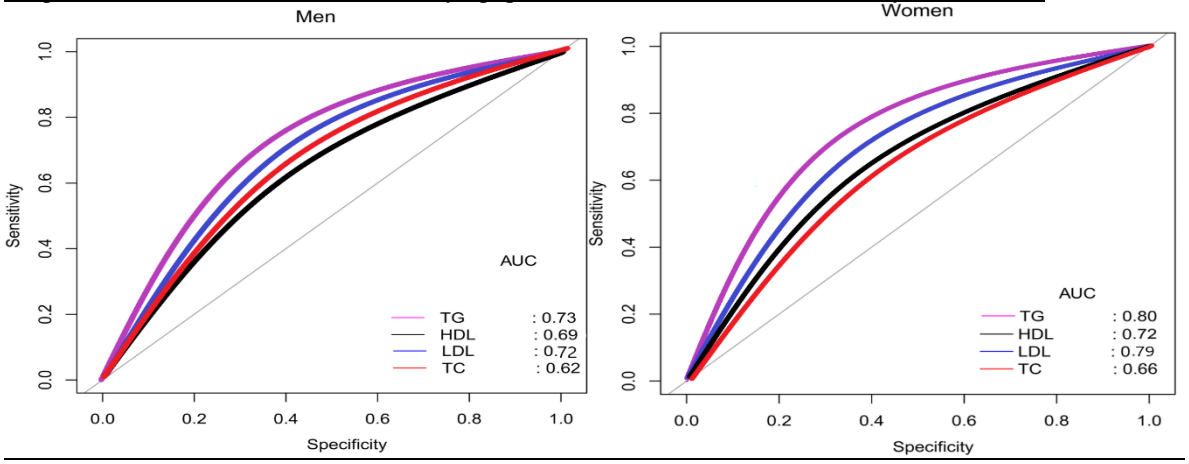
Figure: Table 1: Univariate Analysis

variables	Values (means)	OR (with 95% CI)	P value
1.Sex		Ref	
-Male	670		0.5883
-Female	370		0.0711
2.Age (years)	64.5 ± 5.86	1.32 (1.31, 1.35)	0.4621
3.Ethnicity -Malay -Chinese -Indian -Others	 -540 -342 -78 -40	Ref	
4.BMI (kg/m2)	26.79 ± 1.75	1.65 (1.61, 1.68)	< 0.0001
5.Smoking status (pack years) -Male -Female	 -42.00 -12.00	Ref	< 0.0001
6.HbA1c%	7.64 ± 0.62	5.97 (5.84, 6.02)	< 0.0001
7.HTN (SBP in mmhg)	156.21 ± 12.47	1.88 (1.68, 1.96)	0.0156
8.Lipid Profile			
TC (mmol/L)	5.84 ± 1.04	1.24 (1.17, 1.28)	< 0.0001
TG (mmol/L)	3.52 ± 0.24	5.65 (5.58, 5.69)	< 0.0001
HDL (mmol/L)	0.88 ± 0.72	1.06 (1.01, 1.10)	< 0.0001
LDL (mmol/L)	4.02 ± 1.04	1.32 (1.26, 1.41)	< 0.0001
9.LFT			
-ALT (U/L)	127.62 ± 2.50	1.41 (1.30, 1.48)	< 0.0001
-ALP (U/L)	89.41 ± 1.72	1.11 (1.08, 1.14)	0.6152
-GGT (U/L)	26.65 ± 8.46	0.08 (0.05, 1.01)	0.3764

Values were expressed as mean (standard deviation) or n (%)

Abbreviations: (OR) odds ratio, (CI) confidence interval, (BMI) body mass index, (HTN) hypertension, (SBP) systolic blood pressure, (TC) total cholesterol, (TG) triglycerides, (HDL) high-density lipoprotein, (LDL) low-density lipoprotein, (LFT) liver function test, (ALT) alanine-aminotransferase, (ALP) alkaline phosphatase, (GGT) gamma-glutamyl transferase

Graph 1: Association between low-density lipoprotein levels and non-alcoholic fatty liver disease



PO5-03
Impact of exercise training on hepatic lipid composition in men with MASLD and impaired glycaemic regulation: a pilot randomised controlled trial

Sundus Malaikah[1 2 3], Scott Willis[1 2], Stephen Bawden[4 5], Aron Sherry[1], Jack Sargeant[2 6], Nicole Coull[2], David Stensel[1 2 7 8], Thomas Yates[2 6], Melanie Davies[2 6], David Webb[2 6], Penny Gowland[5], Guruprasad Aithal[4 9], James King[1 2]
[1]Loughborough University, School of Sports, Exercise and Health Sciences, Loughborough, United Kingdom, *[2]NIHR Leicester Biomedical Research Centre, University Hospitals of Leicester and the University of Leicester, Leicester, United Kingdom*, *[3]Clinical Nutrition Department, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia*, *[4]NIHR Nottingham Biomedical Research Centre, Nottingham University Hospitals NHS Trust and the University of Nottingham, Nottingham, United Kingdom*, *[5]Sir Peter Mansfield Imaging Centre, Uiversity of Nottingham, Nottingham, United Kingdom*, *[6]Leicester Diabetes Centre, University of Leicester, Leicester, United Kingdom*, *[7]Faculty of Sport Sciences, Waseda University, Tokyo, Japan*, *[8]Department of Sports Science and Physical Education, The Chinese University of Hong Kong, Hong Kong*, *[9]Nottingham Digestive Diseases Centre, School of Medicine, University of Nottingham, Nottingham, United Kingdom*
Email: s.malaikah@lboro.ac.uk

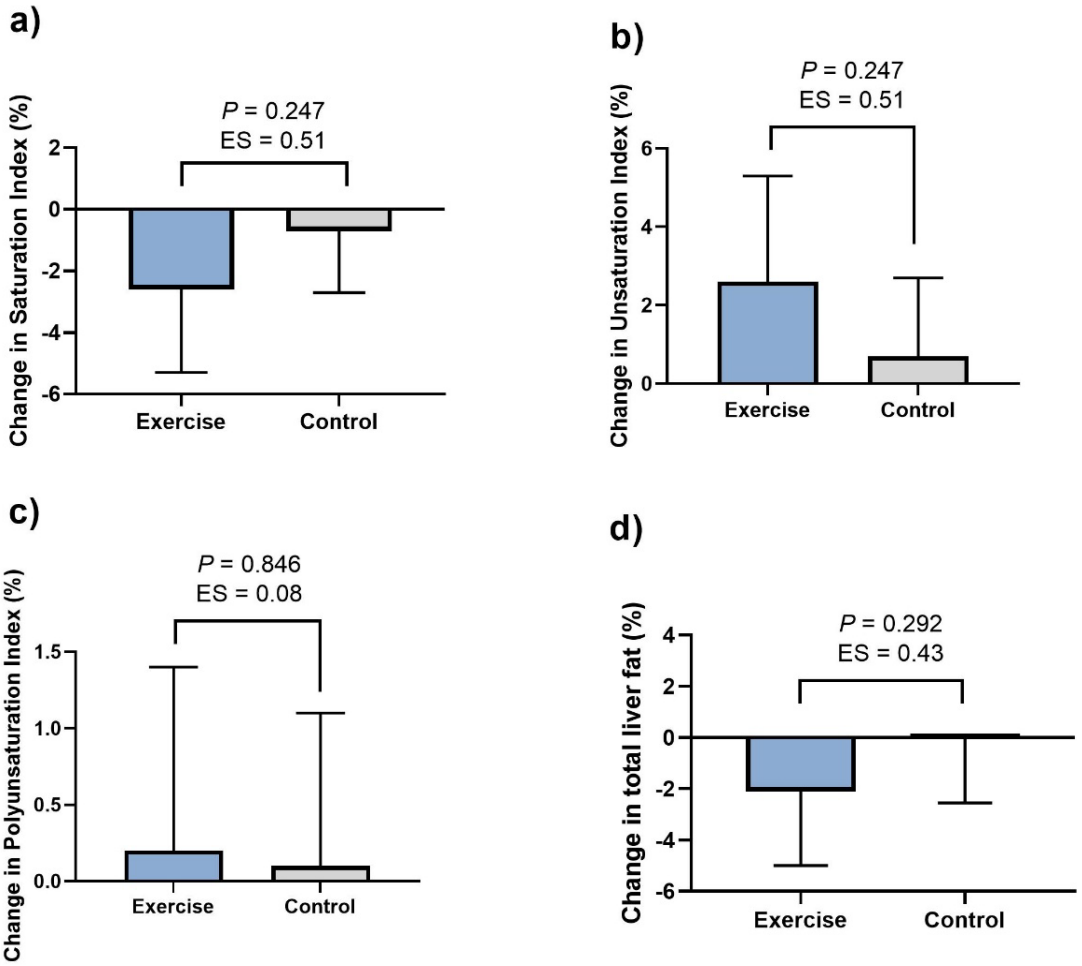
Background and aims: Excessive liver fat is the hallmark feature of MASLD, however, emerging research suggests that liver fat composition may better discriminate associated health risk. Exaggerated saturated hepatic lipids, and depleted polyunsaturated lipids, have been identified in people with MASLD; which is associated with peripheral insulin resistance. One study found that hepatic polyunsaturated lipids were elevated after seven consecutive days of brisk walking, but the impact of exercise training requires closer scrutiny. In a randomised controlled trial (NCT04004273), we examined the impact of 6-weeks of aerobic exercise training on hepatic lipid composition in people with MASLD and impaired glycaemic regulation.

Method: Twenty six men with MASLD and impaired glycaemic regulation (age: 60.3 ± 9.2 years, BMI: 33.5 ± 3.7 $kg \cdot m^{-2}$, liver fat: 17.2 ± 7.4 %, HbA1c: 50.5 (8) $mmol \cdot mol^{-1}$) were randomly allocated to a six-week exercise training intervention or control, in which usual activity and dietary habits were maintained. Exercise training consisted of four moderate-intensity (70 to 75% of age-predicted maximum heart rate) sessions of brisk walking or cycling per week (at least one supervised). The primary outcomes were changes in hepatic lipid composition indices of saturation, unsaturation, and polyunsaturation measured by proton magnetic resonance spectroscopy (^{1}H-MRS; 3.0T Phillips, Ingenia). Secondary outcomes include liver fat, anthropometric measures, and clinical biomarkers. Data were analysed using generalised-linear models adjusted for baseline values and ethnicity. For primary outcomes, simple within group responses were also examined given the pilot nature of this trial.

Results: Twenty-four participants completed the intervention (exercise n = 11, and control n = 13). Primary analysis showed that changes in hepatic lipid composition were similar between the exercise and control groups (Figure 1; a-c). Within-group analysis revealed that the hepatic saturation index was reduced, and unsaturation index increased, after exercise training (both p = 0.022; ES = 0.98). The liver fat response was similar between groups (Figure 1; d). Fasting glucose (-1.2 $mmol \cdot L^{-1}$, p <0.001) and HOMA-IR (-5.1 AU, p = 0.032) were reduced after exercise. Cardiorespiratory fitness (1.5 $ml \cdot kg^{-1} \cdot min^{-1}$, p = 0.013) and waist circumference (-1.8 cm, p = 0.036) were also improved by exercise training. Greater improvements in cardiorespiratory fitness were inversely associated with changes in saturated hepatic lipids (r = -0.522, p = 0.009) and positively associated with changes in unsaturated and polyunsaturated hepatic lipids (r = 0.522, p = 0.009 and rho = 0.543, p = 0.006; respectively).

Conclusion: This trial found that six-weeks of aerobic exercise training did not alter hepatic lipid composition in men with MASLD and impaired glycaemic regulation. However, the magnitude of treatment response and within-group analyses imply that an adequately powered trial is needed to scrutinise this outcome more closely.

Figure: Effect of six weeks of exercise training (exercise) or control on a) saturation index, b) unsaturation index, c) polyunsaturation index, and d) total liver fat. Data are presented as adjusted mean changes and 95% CI. All analyses are adjusted for ethnicity and baseline value of the outcome.



PO5-04
Assessing hepatocellular senescence in metabolic dysfunction-associated liver disease using a quantitative approach

Efstratios Chatzieleftheriou[1], Roberta Forlano[1], Hiromi Kudo[1 2], Mark Thursz[1], Pinelopi Manousou[1], Robert D. Goldin[2]
[1]*Imperial college London, Department of Metabolism, Digestion and Reproduction*, [2]*Imperial college London, Department of Cellular Pathology*
Email: r.forlano@imperial.ac.uk

Background and aims: Metabolic dysfunction-associated liver disease (MASLD) represents an increasing cause of chronic liver disease worldwide. Recent research has linked MASLD with cellular senescence, a state of cell cycle arrest. Liver histology is the gold standard in assessing disease severity, although the evaluation by histopathologists is proven to be subjective. Artificial Intelligence (AI) tools are emerging as solutions, offering more reproduceable assessments. This study aimed to assess factors associated with hepatocellular senescence in MASLD and, using paired liver biopsies, examine changes in senescence alongside disease progression.

Method: Paired biopsies were retrieved from twenty-five patients with a diagnosis of MASLD. Haematoxylin and Eosin (HandE) staining, and immunohistochemistry for p16 (a senescence biomarker) were used. The HandE slides were scored by a histopathologist, and analysed by AI machine-learning for quantitation of fat%, inflammation% and ballooning%. Slides stained for p16 were also quantified using image analysis (Figure 1). Clinical data and non-invasive markers of fibrosis (liver stiffness measurements, LSM) were collected within six months from each biopsy.

Results: In this study, fifty biopsies (25 paired) were included. At the time of the first biopsy, median age was 50 (41-59.5) years and BMI 30.8 (27.7-36.4) kg/m^2, with a median interval between biopsies of 36 (12-84) months. Overall, those with increased p16 expression (defined by the upper quartile cut-off of 3.18%) showed significantly higher inflammation% (5.9 vs 2.2%, p = 0.04), and had higher fibrosis stage F≥3 (p = 0.013) but less nuclear vacuolation (p = 0.006). Moreover, p16 correlated with increased LSM (16.5 vs 9.2 kPa, p = 0.036) and FIB-4 (p = 0.04).When paired liver biopsies were considered, a decrease in inflammation% was significantly associated with decreased p16 expression (p = 0.022). A trend was observed between changes in p16 and changes in FIB-4 and LSM, although this did not reach statistical significance (p > 0.05). Changes in p16 did not correlate to weight loss in this cohort.

Conclusion: Hepatocellular senesce is closely linked to liver disease severity and activity in MASLD. Further research is warranted to investigate senescence as a potential pharmacological treatment target.

Figure:

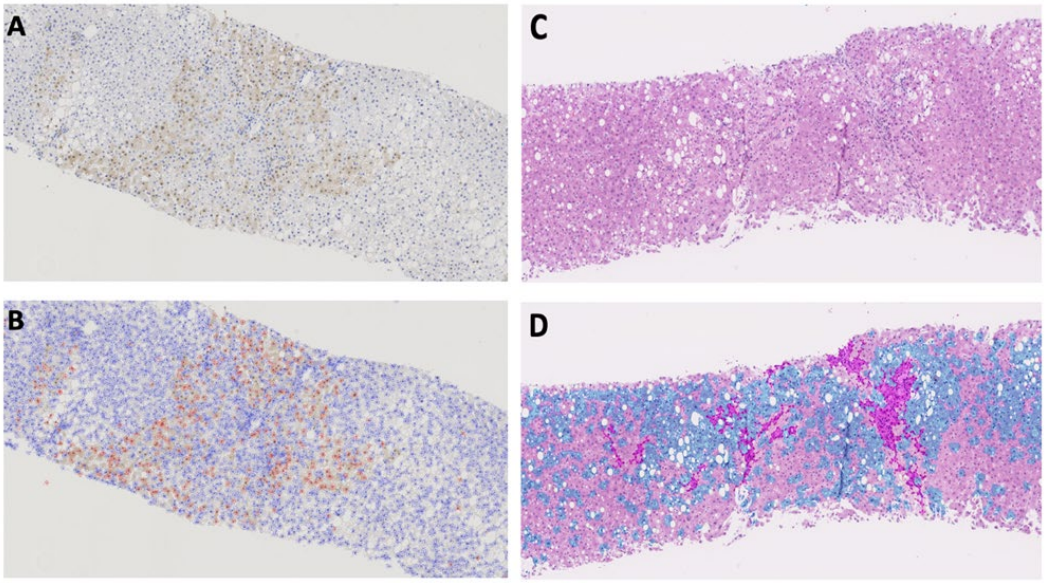


Figure 1. Liver biopsies stained for p16 (A) and Haematoxylin & Eosin (C). When analysing the p16, positively stained nuclei were outlined in red, while negative nuclei were outlined in blue (B). Features of steatosis (in green), ballooning (in blue) and inflammation (in purple) were detected on the H&E slides (D).

PO5-06
Metabolic dysfunction-associated fatty liver disease (MAFLD) and metabolic dysfunction-associated steatotic liver disease (MASLD) are similar definitions to identify patients with fatty liver disease independently of the diagnostic criteria used

Mariana Ramírez-Mejía[1 2], Carlos Jimenéz-Gutiérrez[3], Jacqueline Cordova[4], Rita Dorantes-Heredia[5], Nahum Méndez-Sánchez[6 7]
[1]Medica Sur Clinic and Foundation, Liver Research Unit, Mexico City, Mexico, *[2]Faculty of Medicine, National Autonomous University of Mexico, Plan of combined studies in medicine (PECEM-MD/PhD), Mexico City, Mexico*, *[3]General hospital "Dr. Manuel Gea González", Mexico city, Mexico*, *[4]General hospital "Dr. Manuel Gea González", Hepatology, Mexico City, Mexico*, *[5]Medica Sur Clinic and Foundation, Pathology, Mexico City, Mexico*, *[6]Medica Sur Clinic and Foundation, Liver Research Unit, Ciudad de México, Mexico*, *[7]Faculty of Medicine, National Autonomous University of Mexico, Mexico City, Mexico*
Email: nmendez@medicasur.org.mx

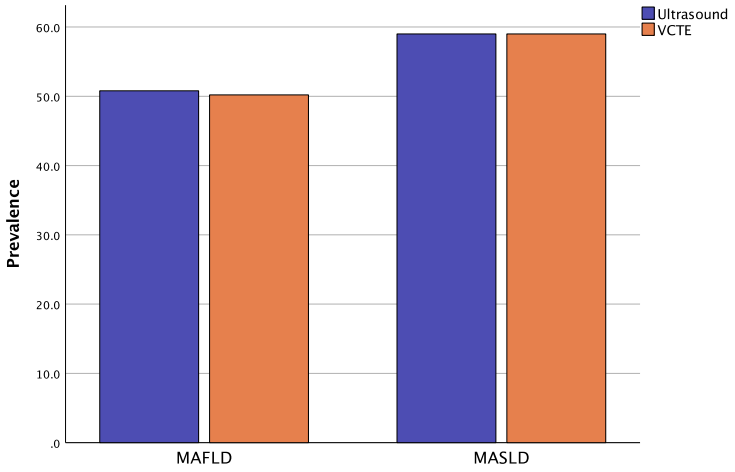
Background and aims: The classification and nomenclature of non-alcoholic fatty liver disease (NAFLD) have been the subject of ongoing debate in the medical community. Through the introduction of metabolic dysfunction-associated fatty liver disease (MAFLD) and the latest release of metabolic dysfunction-associated steatotic liver disease (MASLD), the limitations associated with NAFLD are intended to be addressed. Both terminologies incorporate the metabolic component of the disease by providing comparable diagnostic criteria that rely on the presence of underlying metabolic risk factors. The crucial step in the diagnosis of MAFLD and MASLD is the determination of hepatic steatosis by using imaging and histological techniques. We aimed to evaluate the clinical impact of applying the MAFLD and MASLD diagnostic criteria, utilizing different imaging methods to evaluate hepatic steatosis and determined the clinical profile of individuals with MAFLD and MASLD respectively.

Method: A retrospective analysis of individuals who had undergone abdominal ultrasound and vibration-controlled transient elastography (VCTE) was performed. We evaluated clinical, anthropometric, and biochemical variables to determine the metabolic profile. We assessed the proportion of individuals fulfilling MAFLD and MASLD criteria when either VCTE or ultrasound was used to determine hepatic steatosis.

Results: The study included a total of 500 participants, with 56.8% (n = 284) males and 43.2% (n = 216) females, with a mean age of 48 ± 10 years. Using the ultrasound, 50.8% (n = 254) were diagnosed with MALFD, while 59% (n = 295) were diagnosed with MASLD. Similarly, using the VCTE, 50.2% (n = 251) were diagnosed with MALFD, and 59% (n = 276) were diagnosed with MASLD. Comparing the proportions of MAFLD diagnosis between ultrasonography and VCTE, we observed that both methods detected 82.9% (n = 208) of individuals with MAFLD with a substantial level of concordance (K = 0.644, p = 0.000). Regarding the proportions of MASLD, both ultrasound and VCTE detected 80.8% (n = 223) of the individuals with MASLD, with a moderate concordance level (K = 0.490, p = 0.000).

Conclusion: We observed similarity in the use of MAFLD and MASLD, as both demonstrate a remarkable ability to identify individuals with hepatic steatosis and metabolic dysfunction. Both MAFLD and MASLD are valuable in diagnosing the disease, although MASLD has a higher patient capture compared to MAFLD, resulting in a higher prevalence rate. Furthermore, our results demonstrate that there is no significant statistical difference between the diagnosis of MAFLD and MASLD by ultrasound or VCTE, confirming the feasibility of using readily available imaging methods to diagnose the disease. The MAFLD and MASLD definitions have demonstrated high clinical utility in medical practice, establishing as very effective and valuable approaches to address this growing public health problem.

Figure: Prevalence of MAFLD and MASLD by using ultrasound and VCTE for the assessment of hepatic steatosis according to the diagnostic criteria of each definition.



PO5-08
Assessment of liver fibrosis in individuals with inflammatory bowel disease and steatotic liver disease using non-invasive tests

Robert Nastasa[1 2], Carol Stanciu[1 2], Ermina Stratina[1 2], Sebastian Zenovia[1 2], Remus Stafie[1 2], Adrian Rotaru[1 2], Ana-Maria Singeap[1 2], Cojocariu Salloum[1 2], Catalin Sfarti[1 2], Irina Girleanu[1 2], Horia Minea[1 2], Stefan Chiriac[1 2], Tudor Cuciureanu[1 2], Cristina-Maria Muzica[1 2], Laura Huiban[1 2], Anca Trifan[1 2]
[1]Universitatea de Medicină și Farmacie „Grigore T. Popa" din Iași, Iași, Romania, *[2]Institute of Gastroenterology and Hepatology, Iași, Romania*
Email: robertnastasa948@gmail.com

Background and aims: Individuals with inflammatory bowel disease (IBD) are at high risk for developing steatotic liver diseas (SLD) and severe liver fibrosis (≥F3). The pathogenesis of liver disease among IBD patients can be explained by disease activity, long-term disease duration, hepatotoxic drugs, dysbiosis of gut microbiota, and metabolic factors. This study aimed to assess the diagnostic accuracy of non-invasive tests in predicting severe liver fibrosis (≥F3) in patients with IBD using vibration-controlled transient elastography (VCTE) as a reference quantification method.

Method: We prospectively enrolled patients with IBD and SLD which have been evaluated using non-invasive tests such as aspartate aminotransferase to platelet ratio index (APRI) score, fibrosis-4 (FIB-4) index, and NAFLD fibrosis score (NFS), in the Gastroenterology and Hepatology Institute Iasi, between December 2021 to July 2022. We calculated the area under the receiver operating curve (AUROC), specificity, sensitivity, negative predictive value (NPV), and positive predictive value (PPV) for each of these biomarkers in the detection of severe liver fibrosis (≥F3) compared with liver stiffness measurements (LSM). All the patients included in our study had SLD, according to a controlled attenuation parameter (CAP) ≥ 274 dB/m.

Results: Among 96 patients with IBD and SLD enrolled with a mean BMI of 23.82 ± 3.62 kg/m^2, 58 (60.4%) were females and 67.8% had ulcerative colitis. According to LSM measurements, 9 (9.4%) individuals had severe liver fibrosis (≥F3) using a cut-off ≥9.7 kPa. A significant correlation was found between LSM measurements and FIB-4 index (r = 0.576), NFS (r = 0.587), and APRI score (r = 0.644) (p <0.001). The FIB-4 index had the highest NPV (92.1%) followed by the NFS score (85.6%). Although, all the biomarkers had relatively low specificity (<80%) and PPV (<75 %). The most important finding of our analysis was that all these biomarkers had relatively high NPV (>85%) and accuracy (>83%) for predicting severe liver fibrosis.

Conclusion: FIB-4 index and NFS score appear to be the most appropriate surrogate biomarkers of VCTE for the barring of severe liver fibrosis in IBD patients. Furthermore, it is possible to increase the PPV and reduce the number of cases with indeterminate results for the identification of severe liver fibrosis in patients with SLD, which would suggest a call for action in patients with IBD for both assessments of SLD and liver fibrosis.

PO5-09
SMAD 3 variant rs10152544 is associated with higher fibrosis grade in metabolic steatotic liver disease (MASLD)

Mariana Pinheiro Xerfan[1], Isabel Veloso Alves Pereira[1], Patricia Zitelli[1], Jose Tadeu Stefano[1], Mário Pessoa[1], Claudia Oliveira[1]
[1]Faculdade de Medicina da Universidade de São Paulo (FMUSP), Gastroenterology, Sao Paulo, Brazil
Email: mpxerfan@gmail.com

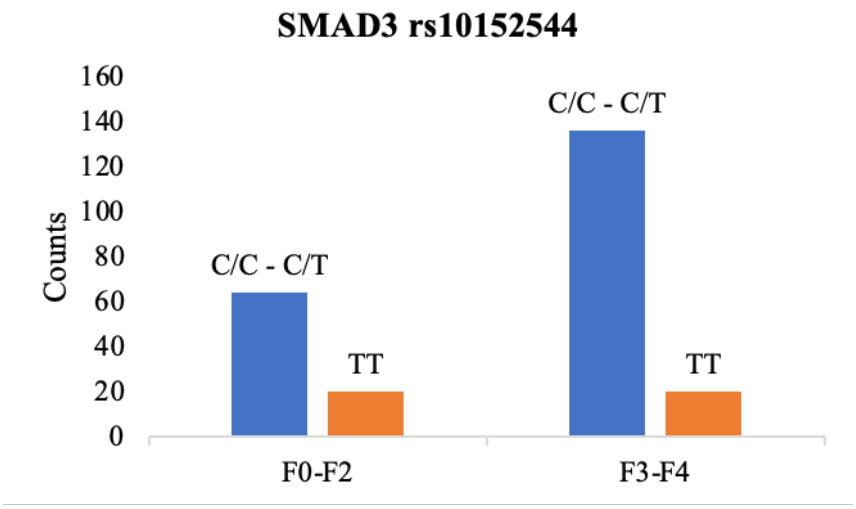
Background and aims: The progression of metabolic steatotic liver disease (MASLD) occurs with the complex interplay between inflammatory stress and lipid accumulation, facilitated by mediators such as pro-inflammatory interleukins and TGF-β1. The intracellular protein SMAD3 participate in the TGF-β1 signaling for fibrosis promoting. Deletion of SMAD3 inhibits type I collagen expression and blocks epithelial-myofibroblast transition. The study aimed to evaluate whether the presence of polymorphisms rs10152544, rs11071932, and rs9806504 in the SMAD3 gene are associated with a higher risk of progression to advanced fibrosis and liver cirrhosis in a Brazilian cohort with biopsy-proven MASLD at a national reference center.

Method: Two hundred and forty saliva samples from patients with MASLD were analyzed. Anthropometric, biochemical, and clinical data were collected retrospectively from the medical record and at the time of sample collection. The fibrosis degree was established by liver biopsy near saliva collection or by patient history combined with elastography results

Results: The study population is mostly female (69.6%, n = 167), with a mean age of 62.8 (± 11.37) and a mean BMI of 31.1 (± 5.65) being classified as obese individuals. Participants are mostly carriers of hypertension and type II diabetes (74.16% (n = 178) and 25.4% (n = 61), respectively). Dyslipidemia was present in 68.3% (n = 164) of individuals. The three comorbidities correlated positively in the sample (p <0.001). The variants frequency was: rs1015254 C/C 27, 9% (n = 67), C/T 55, 4% (n = 133), T/T 16, 7% (n = 40); rs11071932 A/A 93, 8% (n = 225), A/G4, 6% (n = 11), G/A 0, 8% (n = 2), G/G 0, 8% (n = 2); rs9806504 C/T 2, 5 % (n = 6), T/T 96, 7% (n = 232) (96, 7%).The study showed that C/C and C/T genotypes of SNP rs10152544 are associated with higher degrees of fibrosis (F3-F4), p = 0.029 (Figure 1). Furthermore, these genotypes are associated with higher LDL values, a greater degree of ballooning in reassessment biopsies, and higher NAS scores. And in lean individuals, these variants are correlated with the presence of hypertension as well as diabetes in obese individuals. In summary, we conclude that C/C and C/T genotypes of SNP rs10152544 are associated with worse prognoses for MASLD and that SMAD3 appears to participate in different metabolic pathways in the pathogenesis of the disease.

Conclusion: In summary, we conclude that C/C and C/T genotypes of SNP rs10152544 are associated with worse prognoses for MASLD and that SMAD3 appears to participate in different metabolic pathways in the pathogenesis of the disease

Figure:


SMAD3 rs10152544
Counts
160
140
120
100
80
60
40
20
0
C/C - C/T
TT
C/C - C/T
TT
F0-F2
F3-F4

PO5-10-YI
Histological features distinguish alcoholic from metabolic dysfunction-associated steatohepatitis

Tessa Ostyn[1], Frederik Nevens[2 3], Schalk van der Merwe[2 3], Jef Verbeek[2 3], Tania Roskams[1], Olivier Govaere[1]
[1]KU Leuven and University Hospitals Leuven, Department of Imaging and Pathology, Leuven, Belgium, *[2]UZ Leuven, Department of Gastroenterology and Hepatology, Leuven, Belgium*, *[3]KU Leuven and University Hospitals Leuven, CHROMETA Department, Leuven, Belgium*
Email: tessa.ostyn@kuleuven.be

Background and aims: Steatotic liver diseases, encompassing alcoholic-related liver disease (ALD) and metabolic dysfunction-associated steatotic liver disease (MASLD), are characterised by the accumulation of fat in the liver and are mediated by different causes. Although ALD and MASLD share similarities in their clinical and pathological features, they exhibit distinct biological profiles. This study aimed to elucidate the histological variations between ALD and MASLD.

Method: 42 non-cirrhotic patients diagnosed at the University Hospitals Leuven were included, comprising 21 cases of alcoholic steatohepatitis (ASH) and 21 cases of metabolic dysfunction-associated steatohepatitis (MASH). Histological analysis was performed on biopsy sections using HandE and picrosirius red staining. Ubiquitin immunostaining was conducted to visualise the Mallory-Denk bodies. Various histological parameters were assessed, including steatosis grade (<5%, 5-33%, 34-66% and >66%), steatosis zonation (1-3), fibrosis stage (F0-F4), hepatocyte ballooning (0-2), presence of mitochondriosis, satellitosis, glycogen nuclei, and characteristics of Mallory-Denk bodies (semi-quantitatively 0-2 and size 0-2). Satellitosis was defined by the presence of polymorphonuclear cells located near ballooned hepatocytes. Statistical analysis was performed using the Chi-squared test.

Results: Comparison between the ASH and MASH groups revealed no significant differences in fibrosis stage, hepatic steatosis stage, steatosis zone, ballooning, and the presence of glycogen nuclei (Table 1). However, the prevalence of satellitosis was significantly higher in the ASH cohort (81%) compared to the MASH cohort (19.0%). In the ASH group, more patients presented with a high quantity of Mallory-Denk bodies (81.0%), primarily characterised by large-sized inclusions (61.9%). Conversely, MASH patients displayed fewer Mallory-Denk bodies (61.9%) and predominantly exhibited small-sized inclusions (52.4%). Furthermore, a higher occurrence of mitochondriosis was observed in the ASH group.

Conclusion: This study provides evidence that satellitosis and mitochondriosis are more commonly associated with ASH. Mallory-Denk bodies in ASH patients are large-sized and found in greater quantities, whereas MASH patients exhibit fewer Mallory-Denk bodies, primarily small-sized inclusions. Further investigation with a larger cohort is necessary to fully comprehend the histological disparities and the potential differential diagnosis between MASH and ASH.

Figure:

	MASH (n = 21)	ASH (n = 21)	p value
Fibrosis stage, n (%)			0.606
F1	3 (14.3)	3 (14.3)	
F2	10 (47.6)	7 (33.3)	
F3	8 (38.1)	11 (52.4)	
F4	0 (0)	0 (0)	
Hepatic steatosis, n (%)			0.321
<5%	0 (0)	2 (9.5)	
5-33%	8 (38.1)	8 (38.1)	
34-66%	10 (47.6)	6 (28.6)	
>66%	3 (14.3)	5 (23.8)	
Zone steatosis, n (%)			0.739
Random	14 (66.7)	15 (71.4)	
Centrolobular	7 (33.3)	6 (28.6)	
Ballooning, n (%)			0.334
Few	9 (42.9)	6 (28.6)	
Many	12 (57.1)	15 (71.4)	
Satellitosis, n (%)			<0.001
Absent	17 (81.0)	4 (19.0)	
Present	4 (19.0)	17 (81.0)	
Type Mallory-Denk body inclusions, n (%)			<0.001
Absent	1 (4.8)	2 (9.5)	
Small sized inclusions	11 (52.4)	3 (14.3)	
Intermediate/mixed	8 (38.1)	3 (14.3)	
Large sized inclusions	1 (4.8)	13 (61.9)	
Amount of Mallory-Denk body inclusions, n (%)			0.002
Absent	1 (4.8)	2 (9.5)	
Few	13 (61.9)	2 (9.5)	
Many	7 (33.3)	17 (81.0)	
Mitochondriosis, n (%)			0.04
Absent	18 (85.7)	12 (57.1)	
Present	3 (14.3)	9 (42.9)	
Glycogen nuclei, n (%)			0.537
Absent	11 (52.4)	9 (42.9)	
Present	10 (47.6)	12 (57.1)	

PO5-11-YI
Exploring the association between quality of life, diet, physical activity, and binge eating disorder in MASLD patients in a tertiary centre of care

Thishana Kirupakaran[1], Margaux Couffon[1], Beatrice Martino[1], Giordano Sigon[1], Benjamin H. Mullish[1], Michael Yee[2], Mark Thursz[1], Roberta Forlano[1], Pinelopi Manousou[1]
[1]*Imperial college London, Department of Metabolism, Digestion and Reproduction*, [2]*Imperial college London, Department of Endocrinology*
Email: r.forlano@imperial.ac.uk

Background and aims: Metabolic dysfunction-associated liver disease (MASLD) is a rapidly growing cause of chronic liver disease, whose main treatment centres around lifestyle modifications and weight loss. However, weight loss remains challenging, with specific barriers to achieve this being only partially explored. Moreover, increasing evidence suggests a higher prevalence of psychiatric comorbidities, including eating disorders, in MASLD patients, making lifestyle changes even more challenging to achieve. In this study, we aimed to explore the association between quality of life, quality of diet, physical activity, and eating disorder in MASLD patients.

Method: Consecutive patients with a clinical or histological diagnosis of MASLD, followed up in the specialist liver clinic at St Mary's Hospital, Imperial College Healthcare Trust, between October 2022 and April 2023 were included. During consultation, four questionnaires were administered: the 36-Item Short Form survey (SF36) for quality of life, Mediterranean Diet Score (MDS) for diet quality, International Physical Activity Questionnaire (IPAQ) for physical activity, and the 7-item Binge-Eating Disorder Screener (BEDS-7) to screen for eating disorder. Quality of life was analysed using the RAND Corporation Medical Outcomes study as reference. Patients demographic and clinical parametric data, and non-invasive markers of fibrosis were collected at the time of the visit.

Results: Overall, 102 patients were enrolled. Compared to the RAND Corporation Medical Outcomes Study, MASLD patients showed lower scores in all five of the quality-of-life domains ($p < 0.05$): physical functioning, emotional well-being, social functioning, pain, and general health. Moreover, those at risk of BED were significantly younger (44 vs 61 years, $p < 0.001$). Of note, a greater adherence to the Mediterranean diet, as per MDS, was associated with better scores in emotional well-being (84.7 vs 51, $p = 0.024$) and in energy/emotions (64.2 vs 41.6, $p = 0.01$). Similarly, those who remained physical active, as per IPAQ, showed better performance in physical functioning (92 vs 50, $p = 0.006$), emotional wellbeing (92 vs 54, $p = 0.029$), energy/emotions (73 vs 45, $p = 0.002$) and general health (61 vs 43, $p = 0.02$). On multivariate analysis, physical activity was independently associated with a better score in 'role limitations due to emotional problems' SF36 domain (OR:1.058, 95%CI [1.003-1.116], $p = 0.037$) and a lower CAP score (OR:0.954, 95%CI [0.911-1.000], $p = 0.049$).

Conclusion: A higher adherence to Mediterranean diet and to physical activity translated into better performance quality of life. In MASLD patients, lifestyle modifications should be encouraged not only for the clinical benefits, but also for their potential benefit on overall wellbeing. A holistic approach should include a multidimensional management of these patients.

PO5-12
Evaluation of non-invasive scores for monitoring liver fibrosis resolution after bariatric surgery

Violeta Raverdy[1], Estelle Chatelain[2], Federica Tavaglione[3 4], Robert Caiazzo[5], Guillemette Marot[2], Stefano Romeo[6 7], Francois Pattou[8 9]
[1]University of Lille, Lille, France., Lille, France, *[2]Univ. Lille, Inria, CHU Lille, ULR 2694-METRICS : Évaluation des technologies de santé et des pratiques médicales, MODAL, F-59000 Lille, France*, *[3]Clinical Medicine and Hepatology Unit, Fondazione Policlinico Universitario Campus Bio-Medico, Rome, Italy.*, *[4]Department of Medicine and Surgery, Università Campus Bio-Medico di Roma, Rome, Italy.*, *[5]University of Lille, Lille, France.*, *[6]Department of Molecular and Clinical Medicine, Institute of Medicine, Sahlgrenska Academy, Wallenberg Laboratory, University of Gothenburg, Gothenburg, Sweden.*, *[7]Department of Cardiology, Sahlgrenska University Hospital, Gothenburg, Sweden*, *[8]Lille University Hospital, Endocrine and Metabolic Surgery, Lille, France*, *[9]Service de chirurgie générale et endocrinienne, Centre Hospitalier Universitaire de Lille, Lille, France.*
Email: francois.pattou@univ-lille.fr

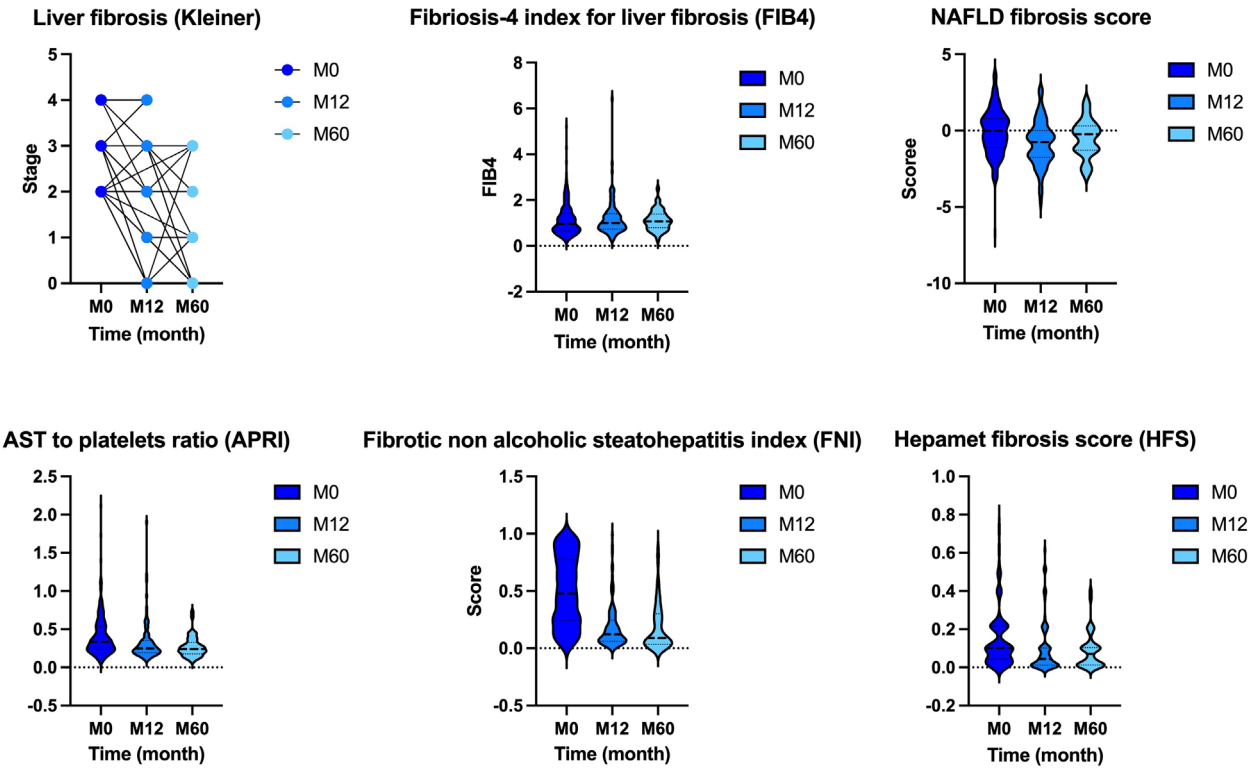
Background and aims: Simple non-invasive scores (NIS) are proposed to replace liver biopsy to diagnose and monitor liver fibrosis in steatotic liver disease. The response to treatment of NIS is however unclear. The aim of the study, we assess the change of NIS in relation with biopsy proven resolution of moderate-to-advanced fibrosis (F≥2), following bariatric surgery (BS).

Method: Participants in this retrospective analysis of a single center prospective cohort study were BS candidates who underwent a liver biopsy and NIS (FIB-4, NFS, APRI, HFS, and NFI), before surgery (M0, n = 2, 523) and/or at one year (M12, n = 626) and/or five years (M60, n =) after surgery. We performed (i) a cross sectional analysis to estimate the AUC of each NIS to diagnose F≥2 (Kleiner, CRN) before and after surgery, and (ii) a longitudinal analysis to estimate (a) NIS change after BS in patients with F≥2 at baseline (covariance pattern model, and effect size/standardized ratio of the mean SRM), (b) AUC of NIS to predict resolution of F≥2 after BS, (c) correlation between change in NIS and Kleiner stage after BS.

Results: (i) NIS AUC for F≥2 diagnosis were >0.7 at M0, M12 and M60 for APRI, HFS and FNI but not for FIB4 (0.69 at M60) and NFS (0.68 at M0 and M12). (ii) (a) Among patients with F≥2 at baseline, liver fibrosis decreased after BS ($p < 0.001$ M12 and M60 vs M0; M60 vs M12) with a large effect size (SRM 0.94 at M12; 1.17 at M60). APR, HFS and FNI significantly decreased after BS ($p < 0.001$, M0 vs M12 and M60) with a moderate to large effect size at M12 (SRM between 0.50 . APRI also decreased from M12 and M60 ($p < 0.05$). NFS decreased at M12 and increased at M60. FIB4 increased after BS. Standardized Response Mean (SRM)

Conclusion: In contrast to FIB4 and NFS, the accuracy of NFI, APRI and HFS to diagnose F≥2 were maintained after BS. Change resolution of moderate to advanced fibrosis after BS. The correlation between individual change in NIS and histological is however limited Our results argue in favor of for a wider use of NIS to follow the evolution of liver fibrosis after BS.

Figure:


Liver fibrosis (Kleiner)
Stage
Time (month)
M0
M12
M60
Fibriosis-4 index for liver fibrosis (FIB4)
FIB4
NAFLD fibrosis score
Scoree
AST to platelets ratio (APRI)
Fibrotic non alcoholic steatohepatitis index (FNI)
Score
Hepamet fibrosis score (HFS)

PO5-13-YI
Early prediction of progressive fibrogenesis in participants with MASLD using blood-based biomarkers

Koen van Son[1], Lars Verschuren[2], Anne-Marieke van Dijk[3], Henrike Galenkamp[4], Arianne van Koppen[2], Sonia Difrancesco[2], Jessica Snabel[2], Anne Linde Mak[3], Bert-Jan van den Born[3], Joanne Verheij[5], Maarten Tushuizen[6], Onno Holleboom[3], Roeland Hanemaaijer[2]
[1]Radboudumc, Gastroenterology and Hepatology, Nijmegen, Netherlands, *[2]TNO, Leiden, Netherlands*, *[3]Amsterdam UMC, Vascular medicine, Amsterdam, Netherlands*, *[4]Amsterdam UMC, Public and Occupational Health, Amsterdam, Netherlands*, *[5]Amsterdam UMC, Pathology, Amsterdam, Netherlands*, *[6]LUMC, Gastroenterology and Hepatology, Leiden, Netherlands*
Email: k.c.vanson@amsterdamumc.nl

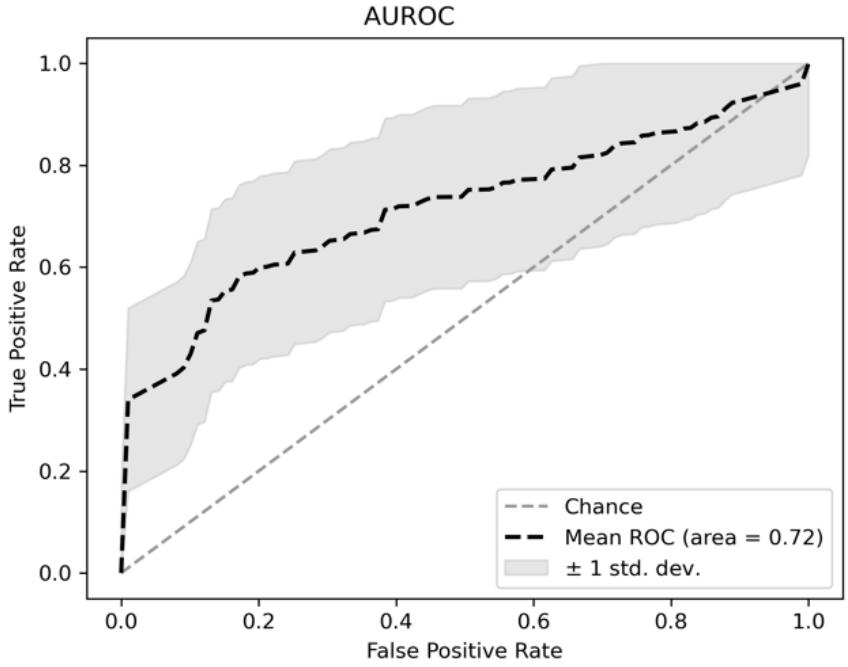
Background and aims: With the increasing prevalence of metabolic-dysfunction associated steatotic liver disease (MASLD), there is a need to identify those patients who develop advanced fibrotic stages. Biomarkers that are directly related to the pathogenesis of hepatic fibrosis, i.e. formation of new matrix proteins, may perform well in identifying those patients who would benefit most from clinical care. Recently, we uncovered a molecular fibrosis signature correlated with the fibrosis stage in participants with histologically characterized MASLD by using biobanked FFPE human liver tissues. This was translated into a set of candidate blood-based biomarkers that are coupled to hepatic extracellular matrix deposition and fibrosis progression. Here we investigate the diagnostic accuracy of the candidate biomarker set to predict future MASLD-related liver stiffness.

Method: The candidate biomarker set for incident MASLD fibrosis was measured in baseline samples from selected participants from the HELIUS cohort from Amsterdam, the Netherlands, with risk factors for MASLD (presence of type 2 diabetes mellitus (T2DM), obesity (BMI ≥30 kg/m^2), elevated waist-hip ratio, Fatty Liver Index (FLI) ≥30, Fibrosis (FIB)-4 ≥1.30 and/or APRI ≥0.42) and compared to Liver Stiffness Measurement (LSM) measured using vibration controlled transient elastrography (VCTE, FibroScan) at six year follow-up. A total of 80 participants were included: 20 with metabolic risk factors (defined as T2DM and/or BMI ≥25 kg/m2) at baseline and LSM ≥9.0 kPa at follow-up, 20 participants with metabolic risk factors at baseline and LSM 7.0-9.0 kPa at follow-up, 20 with metabolic risk factors at baseline and LSM <7.0 kPa at follow-up and 20 without metabolic risk factors at baseline and LSM <7.0 kPa at follow-up. Recursive feature elimination was used to identify the most important biomarker to predict MASLD-related liver stiffness at six years follow-up, defined as LSM ≥7.0 kPa. Sensitivity, specificity and AUROC were calculated for the biomarkers to at baseline predict liver stiffness at six years follow-up.

Results: At baseline median age was 58.0 [48.8; 67.2] years, median BMI was 30.8 [24.8; 34.2] kg/m^2 and 25.0% had T2DM. A total of 52.5% of participants were women. Recursive feature elimination identified one biomarker, a macrophage receptor, that at baseline predicted incident increased liver stiffness at six years follow-up with an AUROC of 0.72, and sensitivity and specificity of 0.72 and 0.62, respectively.

Conclusion: Here we investigate the prognostic potential of a set of blood-based biomarkers coupled to active fibrogenesis, with elastographic liver stiffness as reference standard. We found that one of these biomarkers predicted incident liver stiffness at six years follow-up. Next step will be the validation of these findings by analyses of changes over time and in larger, independent and histologically characterized cohorts.

Figure: AUROC of the biomarker to predict fibrosis at six years follow-up



PO5-14-YI
Impact of a 6-month dietary intervention on cognitive dysfunction and liver status in overweight/obese patients with metabolic dysfunction-associated steatotic liver disease

Nuria Pérez Diaz del Campo[1], Gabriele Castelnuovo[1], Chiara Rosso[1], Matteo Papurello[1], Gian Paolo Caviglia[1], Mariagrazia Tatoli[1], Eleonora Dileo[1], Marta Guariglia[1], Angelo Armandi[1 2], Irene Poggiolini[1], Giorgio Maria Saracco[1 3], Simona Bo[1], Elisabetta Bugianesi[1 3]
[1]University of Turin, Department of Medical Sciences, Torino, Italy, *[2]University Medical Center of the Johannes Gutenberg-University, Metabolic Liver Disease Research Program, I. Department of Medicine, Mainz*, *[3]Città della Salute e della Scienza—Molinette Hospital, Gastroenterology Unit, Turin*
Email: nuria.perezdiazdelcampo@unito.it

Background and aims: Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), the new term for non-alcoholic fatty liver disease, is associated with psychiatric complications, such as depression, anxiety and cognitive impairment, which have a major impact on public health. Lifestyle modifications can help prevent and manage MASLD while also reducing the risk of cognitive dysfunction. In this study, we aimed to assess the impact of a 6-month nutritional intervention on cognitive impairment and liver steatosis in overweight/obese patients with MASLD

Method: 60 patients with MASLD were randomly assigned to one of the three dietary arms: low carbohydrate diet (LcD), Mediterranean diet (MeD) and control diet (CD). The LcD diet consisted of 35-40% carbohydrate, 25-30% protein, 30-35% fat, while the MeD consisted of 50-60% carbohydrate, 15% protein, 25-35% fat. The CD group received general recommendations for a correct lifestyle, similar to those given in clinical practice. Subjects were evaluated at baseline and after 6 months Liver steatosis was evaluated by Controlled Attenuation Parameter (CAP) (Fibroscan®530, Echosens). The cognitive function was assessed by the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS).

Results: Overall, the median age was 52 years (range 42-61) and 66.6% of patients were male. Body mass index and diastolic blood pressure significantly decreased after the intervention (30.28 kg/m^2 *vs*. 28.9 kg/m^2, $P < 0.001$ and 80 mmHg *vs.* 78 mmHg, $P = 0.003$), as well as CAP and insulin values (299.1 dB/m *vs*. 273.5 dB/m, $P < 0.001$ and 10.2 mIU/L *vs* 8.7 mIU/L, $P = 0.028$, respectively). In addition, delayed memory and immediate memory significantly improved their score when comparing baseline vs 6-month results (89.5 *vs.* 95.0, $P = 0.001$ and 84.5 *vs.* 92.3 $P < 0.001$). Interestingly, change in CAP was significantly associated with improvement in immediate memory (r = -0.306, $P = 0.017$) and sum score (r = -0.257, $P = 0.047$). Specifically, patients who decreased their CAP values (N = 46, 76.6%) after the intervention showed an improvement in both delayed (89.5 *vs.* 95, $P = 0.009$) and immediate memory (84 *vs*. 94, $P < 0.001$).

Conclusion: Our results suggest that a 6-month intervention improved cognitive dysfunction, mainly throughout delayed and immediate memory modulation. *This research has been supported by the Italian MIUR under the programme "Dipartimenti di Eccellenza 2018-2022", project code D15D18000410001 and by the University of Torino, grant number ROSC_RILO_21_01.*

PO5-15-YI
Metabolomics analysis showed increased metabolites and acylcarnitine concentrations before and after a lipid load in subjects with steatotic liver disease

Samantha Pezzica[1 2], Fabrizia Carli[1 2], Chiara Rosso[3], Silvia Sabatini[1], Patrizia Infelise[1 2], Angelo Armandi[3], Elisabetta Bugianesi[3], Amalia Gastaldelli[1]
[1]Institute of Clinical Physiology, National Research Council, CNR, Pisa, Italy, *[2]University of Siena, Biochemistry and Molecular Biology, Siena, Italy*, *[3]Division of Gastroenterology, Department of Medical Sciences, A.O., University of Torino, Torino, Italy*
Email: samantha.pezzica@gmail.com

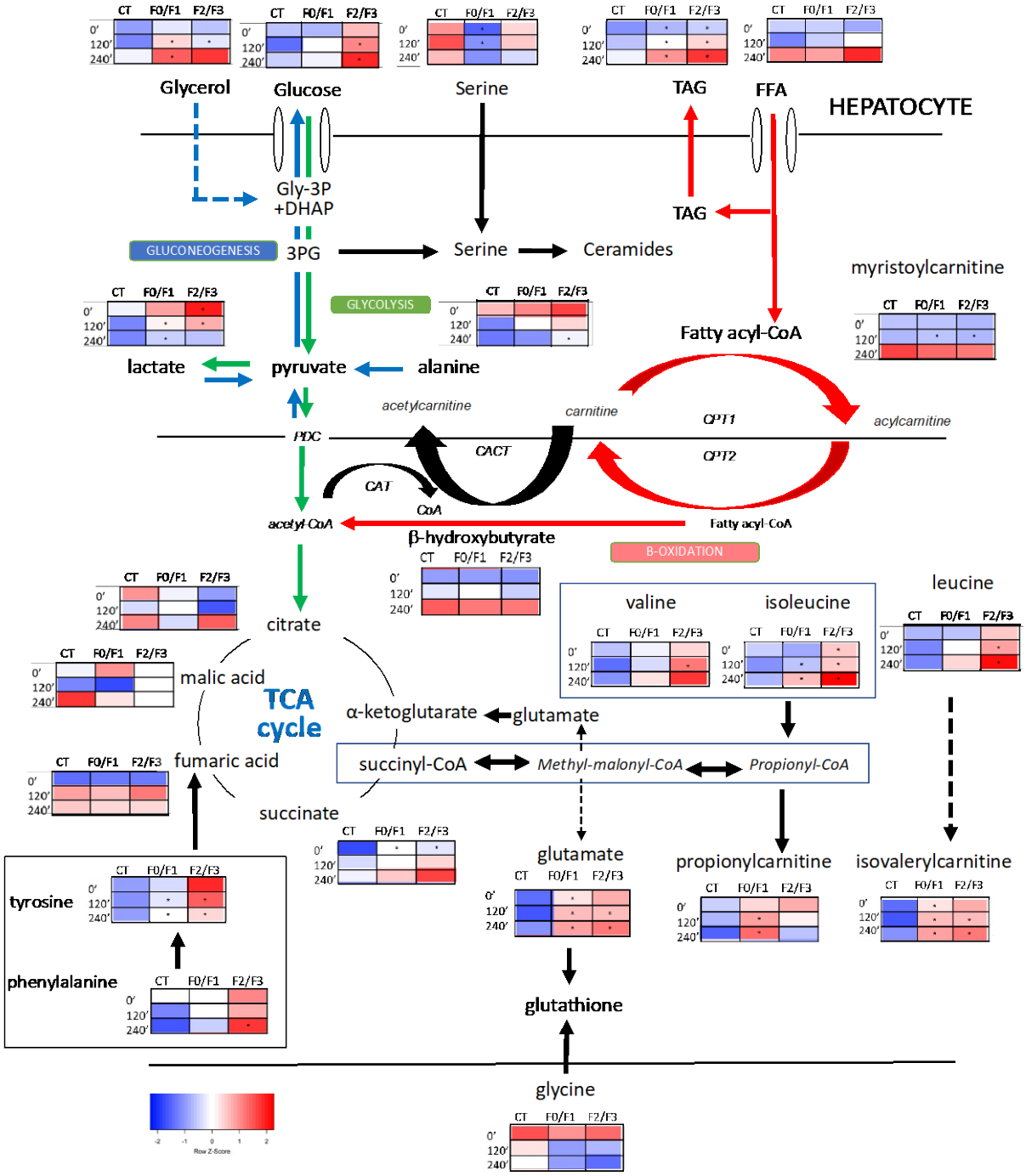
Background and aims: It is well established that severity of steatotic liver disease (SLD) is associated with worsening of insulin resistance and lipidomic profile, but less is known about the changes in metabolites, especially in response to nutrient challenges. Thus, our aim was to investigate how a lipid load impacts not only lipids but also glucose fluxes as well as the release of metabolites associated with energy metabolism in subjects with SLD.

Method: We have studied the metabolomic profile in 20 subjects with biopsy proven SLD (18 male; median age 41 years, BMI 26.7 ± 4.6 kg/m2) with different stage of fibrosis and 8 healthy controls (CT). The analyses have been performed during fasting (t = 0min) and after a lipid load (200ml dairy cream and one egg yolk) followed for 4 hours. The analyses included glucose and lipid fluxes measured by infusion of tracers, metabolomic profile measured by GC-MS. Subjects were grouped according to degree of fibrosis as F0/F1 vs F2/F3.

Results: Figure shows the changes in metabolite concentrations at t = 0, 120 and 240 min in subjects with SLD with F0/F1 or F2/F3 vs CT. At t = 0 (fasting) BCAA, succinate, glutamate and GSG index = glutamate/ (serine + glycine) were significantly increased in F0/F1 and further increased in F2/F3, with concomitant reduction of serine and glycine, consistent with the fibrotic profile. Acyl-carnitines that transport FFA into the mitochondria and beta hydroxybutyrate, which is a marker of FFA hepatic oxidation, were increased from CT to F0/F1 to F2/F3. Triacylglycerol (TAG) and lactate concentrations were significantly higher in patients with F≥2 while glucose, glycerol and alanine levels were similar in SLD and CT. In response to lipid load carnitines, TAG, FFA, glycerol, several amino acids and GSG index increased compared to fasting and were higher in F0/F1 and further in F2/F3 vs CT while glucose concentration was higher in F2/F3 than CT at 240min.

Conclusion: SLD not only had higher concentrations of lipids but also of metabolites that further increased after the lipid load. The respective acyl- and acetyl-carnitines increased too, based on the lipids and amino acids alteration.

Figure:



Metabolites of interest and their changes across the groups (CT vs SLD F0-F1, SLD F2-F3), at time 0, 120' and 240' after lipid load. Data were mean-centered and scaled to standard deviation equal to 1. Abbreviations: CAT, carnitine acetyltransferase; CACT, carnitine acylcarnitine translocase; CPT1, carnitine palmitoyltransferase1; CPT2, carnitine palmitoyltransferase2; PDC, pyruvate dehydrogenase complex. *p value<0.05 vs CT at the same time.

PO5-16
The role of genetic polymorphism in predicting of metabolic associated fatty liver disease clinical course

Vasyl Prysyazhnyuk[1], Oleksandr Voloshyn[1], Iryna Prysiazhniuk[1]
[1]Bukovinian State Medical University, Ukraine
Email: prysyaznyuk_v@ukr.net

Background and aims: Besides the widely known predictors of metabolic associated fatty liver disease (MAFLD) development, like overweight and obesity, diabetes mellitus and insulinresistance, genetic factors could potentially play a role in the development and progression of this disease. The aim of the study was to investigate the role of genetic polymorphism in predicting of the MAFLD clinical course.

Method: The A313G polymorphism of the glutathione-S-transferase P1 (GSTP1) gene with deletion polymorphism of GSTT1 and GSTM1 genes and Pro12Ala polymorphism of the peroxisome proliferation activator receptor-γ (PPAR-γ) gene were studied in 104 MAFLD patients and 45 healthy individuals (control group). Wide spectrum of biochemical parameters was investigated and correlation analysis with particular genotype was performed. Written informed consent to participate in the study was obtained from all the participants.

Results: The study of the possible difference in the prevalence of the A313G allelic variants of the GSTP1 gene polymorphism showed that the G allele occurs significantly more often in MAFLD patients as compared to healthy individuals ($\chi 2 = 5.69$, $p = 0.017$). Carriers of the G allele of the GSTP1 gene (A313G) had a higher activity of alanine aminotransferase as compared to patients with the AA genotype. A higher content of interleukin 10 in the blood of homozygous carriers of the G allele was observed in comparison with patients with AA and AG genotypes. A higher level of atrial natriuretic propeptide, leptin and a lower content of adiponectin in the blood were noted in patients with AG and GG genotypes as compared to patients with AA genotype. Meanwhile no significant difference was found in indicators reflecting the main functions of the liver and markers of its damage in patients with different variants of the deletion polymorphism of GSTT1 and GSTM1 genes. A higher content of tumor necrosis factor-α in the blood was noted in patients with null genotype of the GSTT1 gene as compared to patients with a normal genotype of the GSTT1 gene. A higher level of leptin in the blood was observed in patients with null genotype of the GSTM1 gene in comparison with the patients with normal genotype of the GSTM1 gene. Ala allele of the PPAR-γ gene in MAFLD patients was associated with significantly higher activity of cytolytic syndrome markers as compared to patients with the Pro/Pro genotype. MAFLD patients carriers of the Ala allele have higher levels of leptin and atrial natriuretic propeptide in the blood as compared to patients with the Pro/Pro genotype.

Conclusion: Among the gene's polymorphisms studied only G allele of the GSTP1 gene was associated with higher MAFLD occurrence. However, variety of associations among different polymorphisms of the studied genes with the laboratory markers of cytolysis, pro- and anti-inflammatory cytokine and adipokine profiles were found pointing to their potential impact on MAFLD clinical course.

PO5-17
Synergistic effect of metabolic dysfunction-associated steatotic liver disease and obesity severity in adolescents on future risk of type 2 diabetes: a cohort study

Resthie R Putri[1], Thomas Casswall[1], Pernilla Danielsson[1], Claude Marcus[1], Emilia Hagman[1]
[1]*Karolinska Institutet, Department of Clinical Science, Intervention and Technology, Sweden*
Email: resthie.putri@ki.se

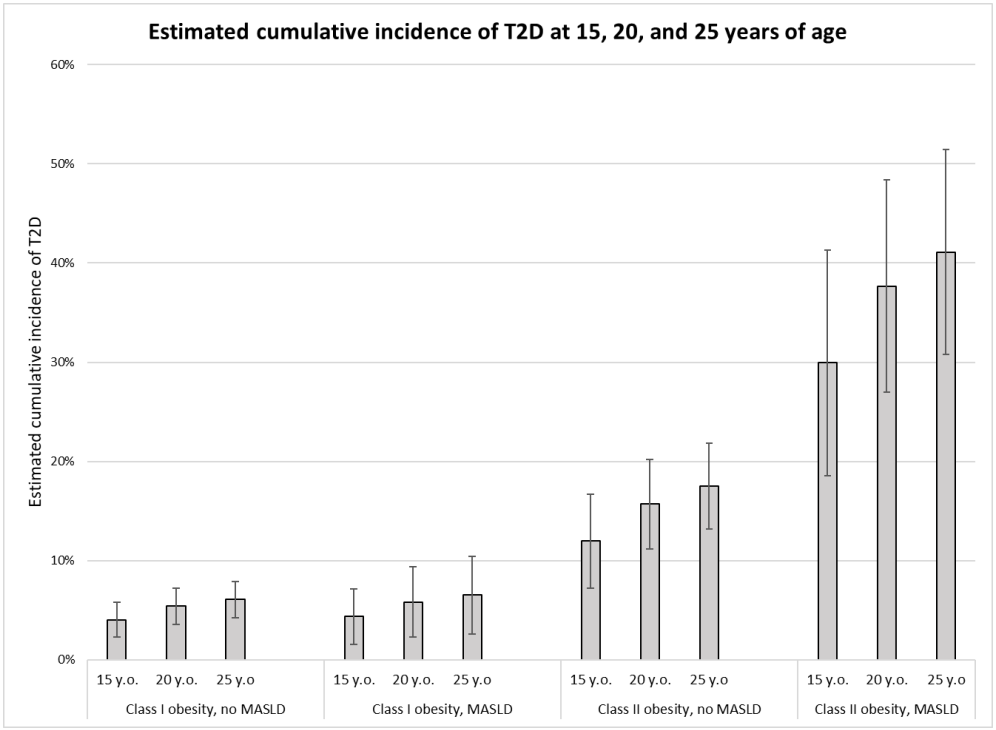
Background and aims: Obesity and metabolic dysfunction-associated steatotic liver disease (MASLD) are risk factors for the development of type 2 diabetes (T2D). We aimed to explore the potential synergistic effect between obesity severity and MASLD among adolescents on future risk for T2D.

Method: In our longitudinal cohort study, we analyzed 3 789 adolescents (aged 10-18) from the Swedish Childhood Obesity Treatment Register (BORIS). The individuals underwent obesity treatment, had two alanine aminotransaminases (ALT) measurement taken at least 6 months apart and were initially free from T2D. Data were linked to national registers. Individuals were followed up to 10 years for the onset of T2D. MASLD was defined as persistent elevated ALT (>44 U/L for females and >52 U/L for males) as marker, without any other discernible causes of steatosis. T2D was identified through ICD 10 codes or prescribed medication. The study population was divided into four groups: 1) class I obesity without MASLD, 2) class I obesity with MASLD, 3) class II obesity without MASLD, and 4) class II obesity with MASLD. Cox regression, adjusted for sex, age, and intermediate hyperglycemia at baseline, was applied.

Results: In the cohort (43% females, 38% severe obesity), 227 individuals (6.0 %) had MASLD at baseline. Over a median follow-up of 4.8 years (Q1-Q3: 2.3-7.9 years), there were 127 cases of T2D onset (median age: 15.8 [13.9-17.6] years). Cumulative incidence of T2D is shown in Figure 1. Compared to those with class I obesity without MASLD, the hazard ratio for developing T2D in class I obesity with MASLD was 1.09 (95% CI 0.26-4.53), in class II obesity without MASLD 3.16 (95% CI 2.11-4.71), and in class II obesity with MASLD 9.08 (95% CI 5.06-16.30). Significant synergistic effect between obesity severity and MASLD on the risk for T2D was observed; Attributable proportion = 0.64 (95% CI 0.38-0.90), indicating that 64% of T2D cases among those with obesity class II with MASLD was attributable to the biological interaction between obesity severity and MASLD.

Conclusion: Among adolescents with obesity, MASLD interacts with obesity severity to synergistically increase the risk of developing T2D. Our results support management of adolescent obesity and MASLD to detect and prevent future metabolic-related disorders.

Figure: Adolescents with class II obesity and MASLD have the highest risk for developing T2D (error bars represent standard error)



PO5-18-YI
Diagnostic accuracy of AGILE3+ score for the non-invasive identification of patients with steatotic liver disease (SLD) and advanced fibrosis: a systematic review and meta-analysis

Andrea Dalbeni[1 2], Rosa Lombardi[3], Matheus Henrique Gonçalves de Souza[4], Mirko Zoncapè[2], Grazia Pennisi[5], Salvatore Petta[5], Ryosuke Tateishi[6], Antonio Colecchia[7], David Sacerdoti[2], Alessandro Mantovani[8], Federico Ravaioli[7 9]
[1]Azienda Ospedaliera Universitaria Integrata Verona, Section of General Medicine C, Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy, Verona, Italy, *[2]Azienda Ospedaliera Universitaria Integrata Verona, Liver Unit, University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy, Verona, Italy*, *[3]Policlinico of Milan, 3 Department of Pathophysiology and Transplantation, Unit of Metabolic and Internal Medicine, University of Milan, Italy., Milano, Italy*, *[4]Universidade Federal do Rio de Janeiro, Department of Internal Medicine, Federal University of Rio de Janeiro, Brazil, Brazil*, *[5]Università degli Studi di Palermo, Sezione di Gastroenterologia, PROMISE, University of Palermo, Italy, Palermo, Italy*, *[6]Graduate School of Medicine and Faculty of Medicine, Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan, Bunkyo City, Japan*, *[7]University of Modena and Reggio Emilia, Gastroenterology Unit, Department of Specialistic Medicines, Modena, Italy*, *[8]Azienda Ospedaliera Universitaria Integrata Verona, Section of Endocrinology, Diabetes and Metabolism, University and Azienda Ospedaliera Universitaria Integrata of Verona, Verona, Italy, Verona, Italy*, *[9]Alma Mater Studiorum-Università di Bologna, Department of Medical and Surgical Sciences (DIMEC), Bologna*
Email: f.ravaioli@unibo.it

Background and aims: Recently, a simple non-invasive score, the Agile 3+ Score, combining liver stiffness measurement (LSM), aspartate aminotransferase/alanine aminotransferase ratio, platelet count, diabetes status, sex and age, has been proposed for identification of advanced fibrosis in patients with suspected Steatotic Liver Disease (SLD). Herein, we performed a systematic review and meta-analysis of observational studies to evaluate the diagnostic accuracy of the Agile 3+ score in identifying patients with SLD and advanced fibrosis.

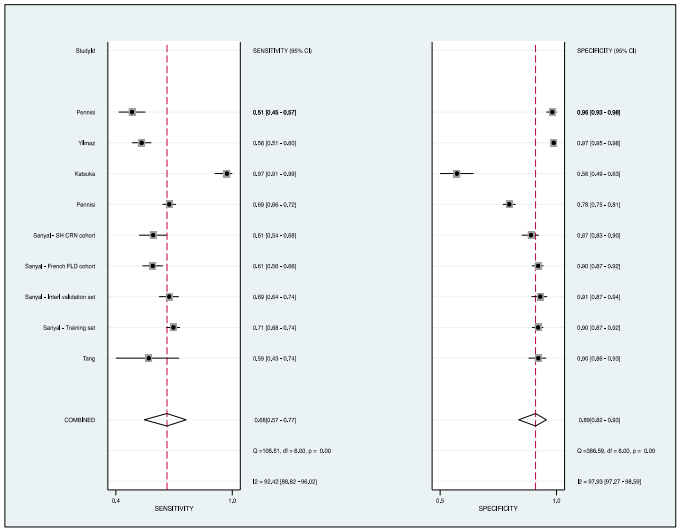
Method: We systematically searched MEDLINE, Ovid Embase, Scopus, and Cochrane Library electronic databases for full-text published articles in any language from the inception to the 24th of April 2023. We included original articles reporting data on the sensitivity and specificity of the Agile 3+ score, according to previously described rule-out (≤0.451) and rule-in (≥0.679) cut-offs.

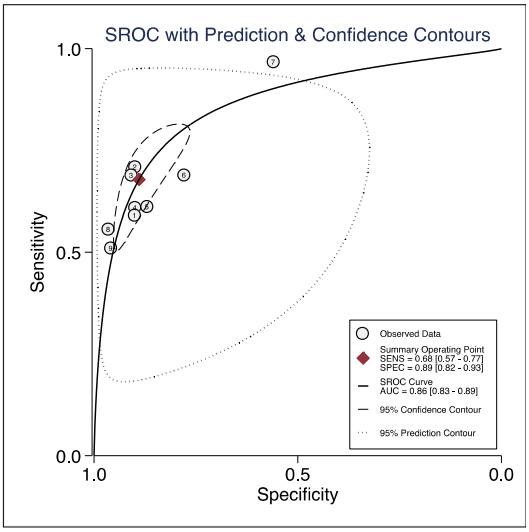
Results: We included 6 observational studies for a total of 8291 participants (mean age 53 ± 5 years; mean BMI 31.4 ± 2.0 Kg/m^2; percentage of men 53.2%, percentage of patients with T2DM 44.9%) with biopsy-proven SLD. The pooled prevalence of advanced fibrosis (≥F3) was 38.2%. By the rule-out cut-off, the overall sensitivity and specificity were 88% (95% CI 84% to 92%; I^2 = 78.5%) and 65% (95% CI 54% to 75%; I^2 = 97.8%), respectively. By the rule-in cut-off, the overall sensitivity and specificity were 68% (95% CI 57% to 77%; I^2 = 92.4%) and 89% (95% CI 82% to 93%; I^2 = 97.9%), respectively. Meta-regression analyses reported that the diagnostic accuracy was partly mediated by BMI values ($p < 0.01$) and sex ($p = 0.04$).

Conclusion: Our systematic review and meta-analysis suggest that the Agile 3+ score has good diagnostic accuracy for the non-invasive diagnosis of SLD/NAFLD and advanced fibrosis. Agile 3+ might be used to identify patients who require a liver biopsy and/or consideration for treatment with emerging pharmacotherapies more efficiently.

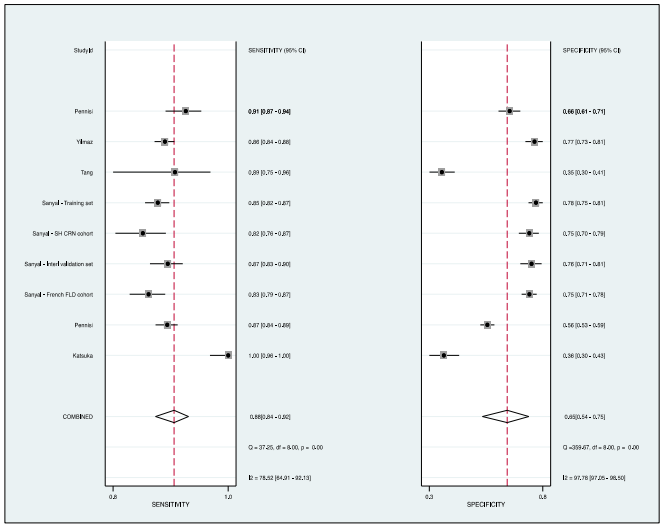
Figure:

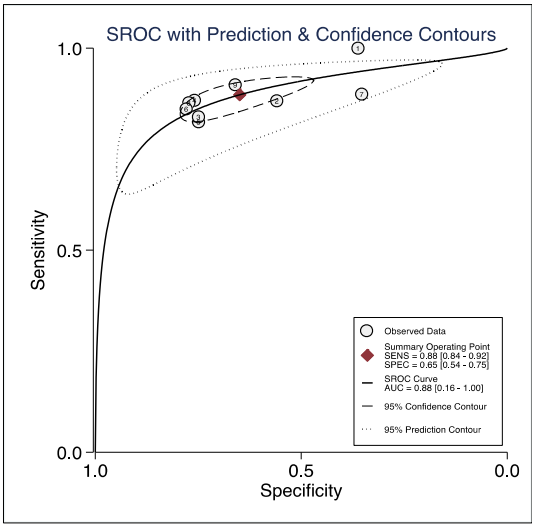
RULE-IN





RULE-OUT





PO5-19
Combining diabetes, sex and menopause as meaningful clinical features associated with NASH and moderate-to-advanced liver fibrosis in unselected individuals with class II and III obesity: a retrospective cohort study

Violeta Raverdy[1], Estelle Chatelain[2], Robert Caiazzo[1], Helene Verkindt[1], Guillemette Marot[3], Francois Pattou[4]
[1]University of Lille, Lille, France., *[2]Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, US 41-UAR 2014-PLBS, billille, F-59000, Lille, France.*, *[3]Univ. Lille, Inria, CHU Lille, ULR 2694-METRICS : Évaluation des technologies de santé et des pratiques médicales, MODAL, F-59000 Lille, France*, *[4]University of Lille, Villeneuve-d'Ascq, France*
Email: francois.pattou@univ-lille.fr

Background and aims: Steatotic liver disease (SLD) is frequent in individuals with obesity. Type 2 Diabetes (T2D), sex, and menopausal status have been identified as independent risk factors for the development of advanced SLD**.** The aim of this study was to combine T2D status, sex, and menopausal status for refining SLD stratification in individuals with severe obesity and gain further insight into disease physiopathology.

Method: We enrolled in a retrospective study 1, 446 participants from the ABOS cohort study (NCT01129297) with class II and class III obesity, who underwent a liver biopsy at the time of bariatric surgery, as well as a comprehensive clinical and biological characterization, including assessment of SLD-related gene expression in the liver. Hierarchical clustering using the binary variables of sex, T2D and menopausal status was applied to classify the study participants. Subsequently, we determined the prevalence of non-alcoholic steatohepatitis (NASH) and moderate-to-advanced liver fibrosis (F≥2) within each identified subgroup, and analyzed the corresponding clinical and biological characteristics.

Results: The prevalence of NASH and F≥2 was respectively 9.5% (N = 138/1, 446) and 11.7% (N = 159/1, 365) in the overall population, 20.3% (N = 107/726) and 21.1% (N = 106/502) in T2D participants and 3.4% (N = 31/920) and 6.1% (N = 53/863) in non-T2D participants. Among T2D participants, NASH and F≥2 prevalence differed in pre-menopausal vs post-menopausal women 15.4% (N = 33/215) vs 30.3% (N = 36/119) (p = 0.002) and 15.5% (32/206) vs 29.5% (33/112) (p = 0.004), respectively. In contrast, NASH and F≥2 prevalence was similar in T2D men above vs below 50 years of age: 21.0% (N = 21/100) vs 18.5% (N = 17/92) and 17.9% (N = 17/95) vs 27.0% (N = 24/89) (p = 0.80 and p = 0.19), respectively. We also found a significant interaction between sex and age with respect to NASH among all T2D patients (p = 0.048) confirming the distinct contribution of menopause to the risk of NASH in T2D women. Finally, we found that several SLD-associated biological traits (lower platelet count; p = 0.001; higher serum uric acid; p = 0.008; gamma-glutamyl transferase; p = 0.02; aspartate aminotransferase; p = 0.029) and liver expressed genes (AKR1B10; fold change: 1.6, p = 0.011 and CCL20; fold change: 1.7, p = 0.019) were significantly associated with menopause in T2D women, but not with age in T2D men.

Conclusion: Overall, these results unveiled a remarkably high prevalence of advanced SLD after menopause in women with T2D, associated with a dysfunctional biological liver profile. This new insight into liver disease heterogeneity merits further exploration.

PO6-01
A 5 sit-to-stand test may identify MASLD patients at higher risk for worse clinical outcomes

Margaux Couffon[1], Thishana Kirupakaran[1], Giordano Sigon[1], Beatrice Martino[1], Jian Huang[1], Benjamin H. Mullish[1], Michael Yee[2], Mark Thursz[1], Roberta Forlano[1], Pinelopi Manousou[1]
[1]Imperial college London, Department of Metabolism, Digestion and Reproduction, *[2]Imperial college London, Department of Endocrinology*
Email: r.forlano@imperial.ac.uk

Background and aims: Metabolic dysfunction-associated liver disease (MASLD) is an important cause of chronic liver disease, with cardiovascular events being the first cause of morbidity and mortality in this population. Sarcopenia has been suggested to predict worse outcomes in cirrhotic patients, however the evidence exploring its impact in non-cirrhotic MASLD patient is scarce. This study aimed to evaluate muscle mass and function in a cohort of MASLD patients and to identify associations with clinical phenotype and disease severity.

Method: Consecutive patients with MASLD were prospectively enrolled at their routine clinical appointment in the liver clinic at St Mary's Hospital (Imperial College Healthcare NHS trust, London). Anthropometric, biochemical, and demographic parameters were collected. Transient elastography was also recorded at the day of the visit. Muscle mass was assessed using fat-free mass index (FFMI) from bioimpedance analysis (BIA). In a small subset of patients, skeletal mass index (SMI) from CT analysis, using machine learning, was calculated. Muscle strength was assessed with the 5 times sit-to-stand test (5STST) and handgrip strength test (HST). Sarcopenia was defined as per guidelines.

Results: A total of 189 patients were included. Low muscle function was found in 64 of 160 (33.9%) as per 5STST, and 41 of 182 (22.7%) as per HST. Low muscle quantity was found in 5.3% (9/169) according to FFMI and 35% (6/17) according to SMI. Patients with poor 5STST showed higher fat mass (%) (39 ± 10.2 vs 33.4 ± 9.3, $p < 0.001$), lower FFMI (55.4 ± 12.1 vs 61.8 ± 12.1, $p = 0.002$), lower muscle mass (kg) (52.77 ± 11.56 vs 58.78 ± 11.61, $p = 0.002$) on BIA, compared to those with normal 5STST. Low SMI and FFMI were significantly associated with lower BMI (26.3 vs 32.7 kg/m2, $p = 0.001$ and 25.6 vs 34.7 kg/m2, $p = 0.004$ respectively). On multivariate analysis, both SMI (OR 0.734, 95%CI: 0.548-0.982, $p = 0.037$) and FFMI (OR 0.803, 95%CI: 0.678-0.952, $p = 0.010$) were independently associated with lower BMI. Moreover, previous history of MACE (OR 14.98, 95%CI: 2.517-89.149, $p = 0.003$), BMI (OR 1.079, 95%CI: 1.004-1.161, $p = 0.04$), LSM (OR: 1.073, 95%CI 1.003-1.148, $p = 0.042$) and male gender (OR 0.276, 95%CI: 0.117-0.65, $p = 0.003$) were independently associated with a poor performance on 5STST. LSM was also independently associated with a poor HST (OR 1.106, 95%CI: 1.038-1.18, $p = 0.002$), even after adjusting for BMI.

Conclusion: Both severity of liver disease and history of cardiovascular events predict sarcopenia in patients with MASLD. A 5 sit-to-stand test is a quick, inexpensive measurement which could assist with identifying a MASLD phenotype who may be at higher risk for worse clinical outcomes.

PO6-03
Association between hepatic artery resistance index (HARI), portal venous pulsatility index (PVPI) and steatotic liver disease (SLD) fibrotic score in SLD patients

Simu Razvan-Ioan[1], Andreea Dobroaia[1], Mircea Istrate[1], Letitia Toma[1], Simona Ioanitescu[1], Elena Laura Iliescu[1]
[1]*Fundeni Clinical Institute, Internal Medicine, Bucharest, Romania*
Email: simu.razvan@yahoo.com

Background and aims: Ultrasound has always been an accessible, quick and reliable non-invasive method used to highlight the presence of steatosis and Steatotic Liver Disease (SLD). However, it cannot evaluate the presence and stage of fibrosis in patients with SLD. The SLD fibrotic score is a non-invasive way to identify and grade liver fibrosis in patients with SLD, taking into account biologic parameters. Hepatic artery resistance index (HARI) and portal venous pulsatility index (PVPI) have previously proven to be useful tools in assessing liver function and damage. We aim to determine whether there is a correlation between the hepatic vascular indexes and the SLD fibrotic score in patients with SLD.

Method: We conducted a single center observational study on patients with SLD with different degrees of fibrosis admitted to our clinic between July 2022 and May 2023. Patients with diagnosed cirrhosis were excluded. We determined the SLD fibrosis score for all patients and evaluated the US aspect of the liver along with the hepatic vessels, RI of the hepatic artery and pulsatility of the portal vein. We divided patients in 2 groups: advanced (F3-F4) and non-advanced (F0-F2) fibrosis.

Results: We evaluated a total of 270 patients of which 152 (56.3%) were male and 118 (43.7%) were female. The mean age of the patients was 55.72 ± 19.72 (range between 36 and 74 years). HARI was higher in patients with advanced fibrosis than in those with low or moderate fibrosis (0.77 vs 0.64) ($p < 0.01$) whereas PVPI was lower (0.4 vs 0.47). We found a positive correlation between increased HARI, decreased PVPI and degree of fibrosis in SLD patients. ($p < 0.02$)

Conclusion: US Doppler may be valuable tool for assessing the presence of liver fibrosis, as both HARI and PVPI indexes appear to correlate with the SLD fibrotic score. We further aim to push and extend the limits of US hepatic vascular indexes and determine their utility in predicting further complications in SLD patients.

Figure:

Fibrosis grade	SLD index	HARI	P value HARI	PVPI	P value PVPI
F0	-1.602±0.10	0.59±0.07	0.03	0.50±0.21	0.02
F1	-1.560±0.14	0.64±0.05	0.02	0.47±0.20	0.02
F2	-1.467±0.11	0.7±0.06	0.04	0.44±0.19	0.01
F3	0.690±0.16	0.76±0.05	0.01	0.42±0.18	0.03
F4	0.820±0.21	0.79±0.07	0.02	0.39±0.15	0.01

PO6-05
Changes in lipid metabolism and insulin resistance after an oral lipid load in patients with metabolic dysfunction associated steatotic liver disease (MASLD) carrying the PNPLA3 rs738409 polymorphism

Chiara Rosso[1], Samantha Pezzica[2], Silvia Sabatini[2], Fabrizia Carli[2], Francesca Saba[1], Gian Paolo Caviglia[1], Patrizia Infelise[2], Marta Guariglia[1], Angelo Armandi[1], Roberto Gambino[1], Elisabetta Bugianesi[1], Amalia Gastaldelli[1]
[1]University of Turin, Department of Medical Sciences, Torino, Italy, *[2]Institute of Clinical Physiology, CNR, Pisa, Cardiometabolic Risk Unit, Pisa, Italy*
Email: amalia.gastaldelli@cnr.it

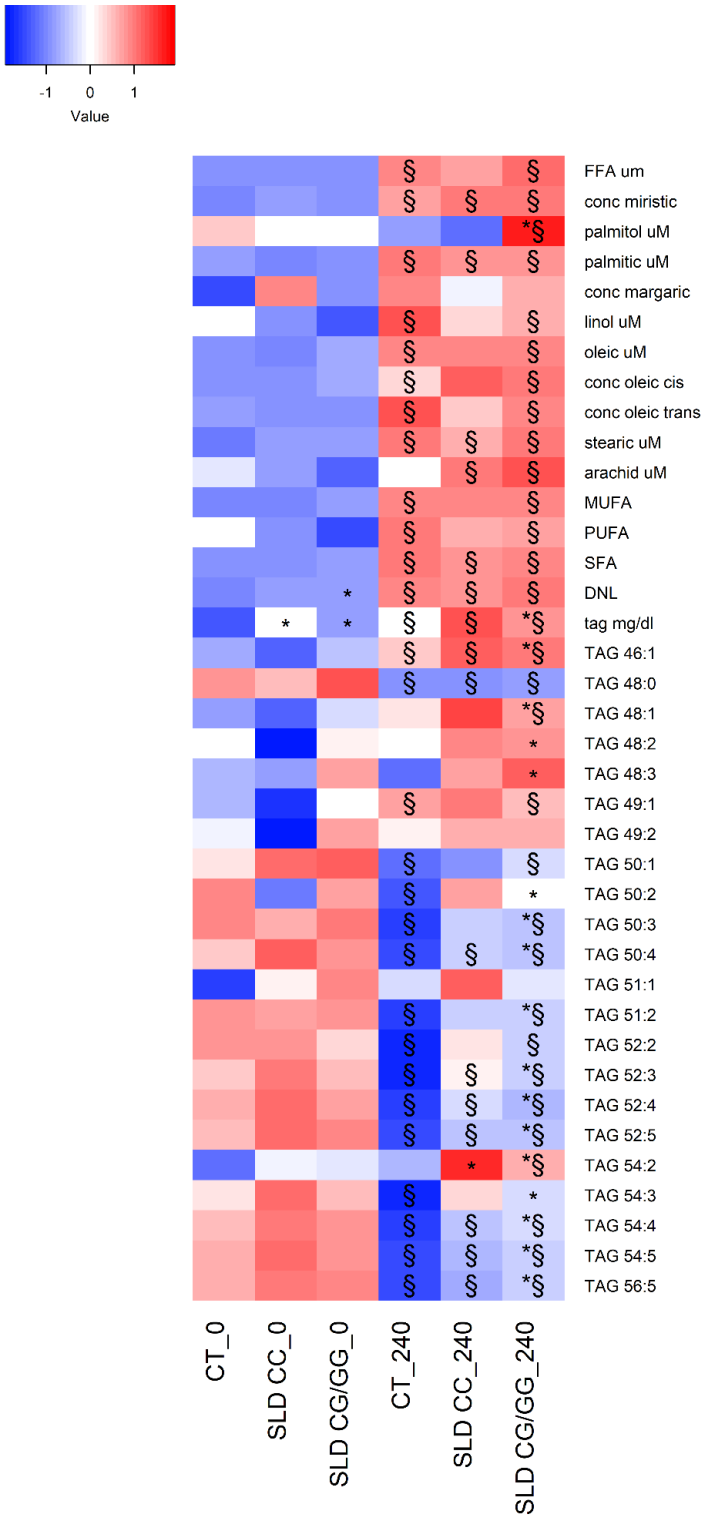
Background and aims:
In subjects with metabolic dysfunction associated steatotic liver disease (MASLD), hepatic fat accumulation is the result of insulin resistance (IR) and the impairment of hepatic glucose and lipid metabolism. In addition, the rs738409 C>G patatin-like phospholipase domain-containing 3 (*PNPLA3*) polymorphism is one of the main predisposing factors for the onset and progression of MASLD and hepatic fibrosis. Our aim was to assess the impact of *PNPLA3* rs738409 polymorphism on lipid metabolism and lipidomics in a group of subjects with biopsy-proven MASLD without diabetes.

Method: Lipid metabolism was studied in 20 subjects with biopsy proven MASLD (18 male; median age 41 years, range: 23-57) and 8 healthy controls (CT) genotyped for *PNPLA3* by allelic discrimination assay. At baseline and after a lipid load (200ml dairy cream and egg yolk), tracers (6, 6-2H2-glucose and 2H5-glycerol) were infused for 6h to evaluate lipolysis and endogenous glucose production [EGP]. Target lipidomics by high-resolution mass spectrometry for triglyceride (TAG) and free fatty acids (FFAs) composition at fasting and in response to the oral lipid load. We calculated DNL index from FFA as C16:1/C16:0 and IR indexes from tracers and insulin (INS) as: hepatic IR (Hep-IR = EGP x INS), adipose tissue IR (Lipo-IR = lipolysis x INS or AT-IR = FFAs x INS).

Results: Prevalence of *PNPLA3* rs738409 G minor allele was 75% (15/20) in MASLD patients and 44% (4/9) in CT (p = 0.116). During fasting EGP, lipolysis and GC were similar in both groups even if NAFLD subjects were more insulin resistant than CT (Hep-IR: 88 vs. 52, p = 0.004; Lipo-IR 24 vs. 13, p = 0.011; AT-IR: 5.1 vs. 4.4, p = 0.043). When we compared MASLD patients carrying the *PNPLA3* G risk allele with those carrying the CC genotype, we showed no differences in fasting EGP, lipolysis, and IR components or FFA and TAG composition. DNL index was higher in CG/GG. In all subjects during the last hour (180-240min) FFAs and TAG concentrations increased and TAG levels were slightly higher in NAFLD subjects who carried the *PNPLA3* CC genotype compared to CG/GG, but the composition was different (figure). Cholesterol profile did not change during meal in CT vs. NAFLD.

Conclusion: MASLD patients who carry the *PNPLA3* rs738409 G minor allele show an altered lipid metabolism and composition after an oral lipid meal. The implications of these results should be further explored. *Funded by Horizon2020 under grant agreement no.634413, EPoS*

Figure: FFA and TAG % composition during fasting (t = 0min) and at the end of lipid load (t = 240min). * t-test p value <0.05 vs CT, § paired t-test pvalue pre *vs*. post <0.05, No statistical differences in *PNPLA3* CC vs CG/GG



PO6-06-YI
Analytical and clinical comparison of three non-invasive tests for the diagnosis of metabolic dysfunction-associated steatohepatitis (MASH)

Silvia Sabatini[1], Giulia Angelini[2], Erminia Lembo[2], Ornella Verrastro[2], Fabrizia Carli[1], Geltrude Mingrone[2 3], Amalia Gastaldelli[1]
[1]Institute of Clinical Physiology, CNR, *[2]Università Cattolica del Sacro Cuore, and Fondazione Policlinico Universitario Agostino Gemelli IRCCS*, *[3]King's College London*
Email: silvia.sabatini2@student.unisi.it

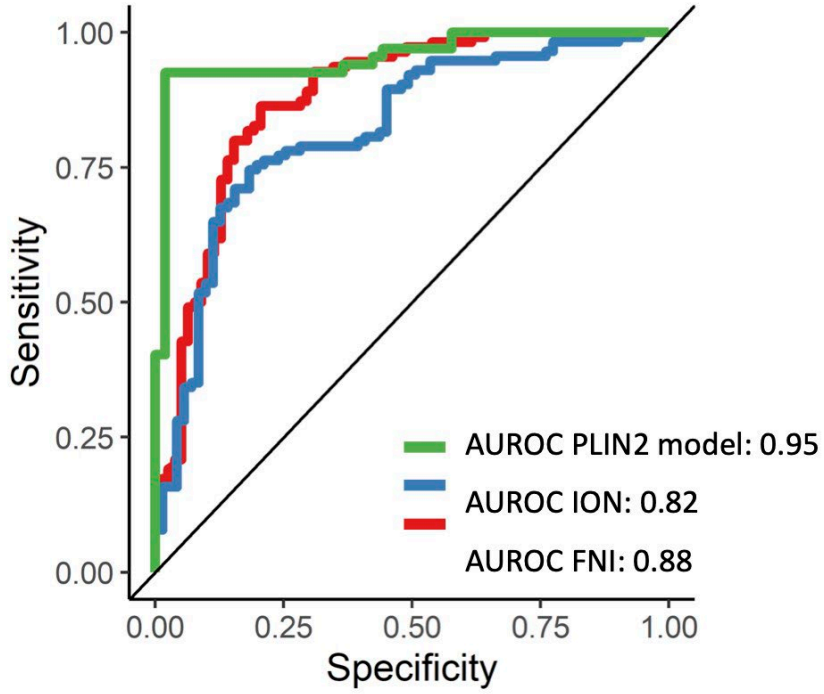
Background and aims: Metabolic dysfunction-associated steatohepatitis (MASH) is the most common liver disease worldwide and the leading cause of liver-related morbidity and mortality. The large number of MASH subjects with potential for progressive liver disease creates challenges for screening, as the diagnosis of MASH currently relies on invasive liver biopsy. Therefore, there is an urgent need to find non-invasive test (NITs) for MASH diagnosis and disease progression. However, until now, no specific biomarker has been officially endorsed by the FDA. Recently, we have developed a model to predict MASH based on PLIN2 mean florescence intensity (MFI) in monocytes combined with waist circumference (WC), triglyceride (TG), alanine aminotransferase (ALT) and presence/absence of diabetes as covariates (HeparDx™ score). Other two NIT for MASH, i.e., the Fibrosis Nash Index (FNI) and the Index Of NASH (ION) were previously proposed but not validated on external cohort with biopsy proven MASH. Thus, we aimed to compare and assess the utility of these tests in two trials with liver biopsies, i.e., the Bariatric Surgery Versus Non-alcoholic Steato-hepatitis-BRAVES trial and the Liquid Biopsy for NASH and Liver Fibrosis-LIBRA trials.

Method: We analyzed data from two trials that included 198 subjects with liver biopsy (BRAVES cohort, n = 119; LIBRA cohort, n = 79) with histologically proven MASH with or without fibrosis or liver steatosis but noMASH. We compared the PLIN2 score (using only the validation cohort) with other two scores for presence of MASH (FNI based on aspartate aminotransferase (ALT), high density lipoprotein (HDL) and HbA1c and ION based on sex, waist/hip ratio, TG, ALT, and HOMA-IR calculated in all subjects).

Results: The cohort included subjects with MASH (n = 117) and noMASH (n = 78) with different histological response, range of BMI (41.96 ± 5.86) and glycemic control (HbA1c = 40.22 ± 7.81). Although all three scores for MASH exhibited high accuracy, the PLIN2 model was the most accurate (AUROC: PLIN2 model = 0.95, FNI = 0.88, ION = 0.85, figure).

Conclusion: The three NIT for MASH are accurate, sensitive, and specific for the diagnosis of MASH. PLIN2 is candidate as NIT of MASH and possible hepatic target although its validity should be further investigated in other cohorts.

Figure:


1.00
0.75
0.50
0.25
0.00
Sensitivity
0.00
0.25
0.50
0.75
1.00
Specificity
AUROC PLIN2 model: 0.95
AUROC ION: 0.82
AUROC FNI: 0.88

PO6-07-YI
The association of liver fibrosis, insulin resistance and systemic leukocyte activation in morbidly obese patients in relation to endothelial damage

Alina Saidi[1], Willy Theel[1], Jan Apers[2], Vivianne de Jong[3], Stefanie van Mil[4], Aart-Jan van der Lelij[4], Diederick Grobbee[3], Ellen van der Zwan-van Beek[5], Manuel Castro Cabezas[1]
[1]Franciscus Gasthuis and Vlietland, Department of Internal Medicine and Endocrinology, Rotterdam, Netherlands, *[2]Franciscus Gasthuis and Vlietland, Obesity Center CGG, Rotterdam, Netherlands*, *[3]Julius Clinical, Zeist, Netherlands*, *[4]Erasmus University Medical Center, Department of Internal Medicine, Rotterdam, Netherlands*, *[5]Franciscus Gasthuis and Vlietland, Department of Clinical Chemistry, Rotterdam, Netherlands*
Email: a.saidi@franciscus.nl

Background and aims: Obesity is strongly associated with metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), and metabolic dysfunction-associated steatohepatitis (MASH). Chronic low-grade inflammation leads to activation and infiltration of leukocytes in the liver potentially leading to injury, inflammation and fibrosis. This study investigated the relation between FIB-4 as a marker of liver fibrosis and systemic leukocyte activation among morbidly obese patients.

Method: Analyses were done using data from morbidly obese patients who were scheduled for bariatric surgery. Systemic leukocyte activation was quantitated by flowcytometry. Relations between FIB-4, systemic inflammation, estimated by leukocyte activation markers, and markers of endothelial damage like carotid intima media thickness (cIMT) and pulse wave velocity (PWV) were assessed.

Results: The total cohort consisted of 200 subjects (148 women, mean age 41 ± 12 years, mean BMI 42.7 ± 5.2 kg/m2). Compared to patients with low FIB-4 scores (<1.3), patients in the elevated FIB-4 group (≥1.3) had higher systolic blood pressure (146 ± 16 vrs 139 ± 16 mmHg, respectively), higher diastolic blood pressure (91 ± 13 vrs 83 ± 12 mmHg), higher cIMT values (0.66 ± 0.1 vrs 0.55 ± 0.1 mm), higher PWV (8.2 ± 0.9 vrs 6.8 ± 1.1 m/sec), higher HbA1c (48 ± 14 vrs 42 ± 12 mmol/mol), and higher glucose levels (7.0 ± 2.9 vrs. 5.9 ± 2.2 mmol/l). Patients with an elevated FIB-4 tended to have increased expression of neutrophils CD66b ($p = 0.082$) and monocyte CD11b ($p = 0.071$) surface markers, suggesting a more pronounced systemic inflammatory state in these patients.

Conclusion: Morbidly obese patients with an increased risk of liver fibrosis had more insulin resistance and signs of endothelial damage based on higher cIMTs and stiffer arteries (PWV). The data of this study suggest that patients at risk of liver fibrosis may be characterized by systemic inflammation reflected by more activated neutrophils and monocytes.

PO6-09-YI

The impact of sarcopenia on both liver and cardiovascular alterations in patients with early stages of steatotic liver disease: a sole protagonist or a co-actor with other metabolic risk factors?

Floriana Santomenna[1 2], Annalisa Cespiati[1 2], Rosa Lombardi[1 2], Matteo Carlomagno[2], Daniel Smith[1 2], Jaqueline Currà[1 2], Giuseppina Pisano[1], Felice Cinque[1 2], Giovanna Oberti[1], Anna Ludovica Fracanzani[1 2]
[1]Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, SC Medicina ad Indirizzo Metabolico, MILANO, Italy, *[2]University of Milan, Department of Pathophysiology and Transplantation, MILANO, Italy*
Email: floriana.santomenna@unimi.it

Background and aims: Sarcopenia is the progressive loss of skeletal muscle mass, function and strength. Metabolic disorders contribute to its onset, as well as to the development of hepatic steatosis. The prevalence of sarcopenia and its association with liver and cardiovascular (CV) alterations in the early stages of steatotic liver disease (SLD) is still underreached. 1) to evaluate the prevalence of sarcopenia detected by bioimpedance analysis (BIA), in a cohort of non-cirrhotic patients with SLD; 2) to evaluate the impact of sarcopenia and metabolic comorbidities on liver and CV alterations in the same cohort.

Method: 483 non-cirrhotic SLD patients (mean age 51 ± 12 ys, 65% male) were enrolled. Liver ultrasound (US) defined the presence and severity of hepatic steatosis. A skeletal muscle mass/height^2 ≤10.75 kg/m^2 and ≤6.75 kg/m^2 by BIA defined sarcopenia in men and women, respectively. Presence of carotid plaques at supra-aortic trunk echo-doppler defined CV damage. Epicardial fat thickness (EFT) at echocardiography ≥9.5 mm in men and ≥7.5 mm in women defined increased visceral adiposity. Fibroscan by liver stiffness measurement (LSM) ≥8 kPa suggested advanced fibrosis.

Results: 39% of the cohort was sarcopenic, while 6% had hepatic fibrosis by LSM≥8 kPa . Mean body mass index (BMI) was 28.9 ± 4.4 kg/m^2, with 83% of the cohort overweight, 43% of patients dyslipidemic, 37% hypertensive, 11% diabetic. Compared to non-sarcopenic, sarcopenic subjects were more prevalently males (47% vs 91%, p < 0.001), had lower BMI (30.3 vs 26.9 kg/m^2, p < 0.001) and waist circumference (WC) (103 vs 99.5, p = 0.003), lower prevalence of increased EFT (37% vs 16%, p <0.001) but the same prevalence of severe steatosis, fibrosis (LSM mean values and >8 kPa) and carotid plaques. At multivariate analysis (adjusted for age, sex, factors associated with sarcopenia at univariate analysis), sarcopenia remained independently associated with lower BMI (OR 0.44, 95% CI 0.34-0.57, p < 0.001), lower WC (OR 0.81, 95% CI 0.75-0.88, p < 0.001), and male sex (OR 14.2, 95% CI 5.67-35-8, p < 0.001). When analyzing different metabolic subgroups of patients, sarcopenic hypertensive subjects compared to non-sarcopenic ones had a higher prevalence of carotid plaques (57% vs 37%, p = 0.02) and mean LSM values (6.8 vs 5.9 kPa, p = 0.05), without any difference in smoking status or dyslipidemia.

Conclusion: Non-cirrhotic SLD patients had a high prevalence of sarcopenia, especially if non-obese and males. Despite the lower visceral adiposity, sarcopenic patients presented the same extent of hepatic steatosis and fibrosis. However, the coexistence of sarcopenia and hypertension had a significant impact on both liver and CV disease, suggesting an additive negative effect. Patients with SLD would need a prompt evaluation and treatment of sarcopenia to prevent the progression of liver and CV disease, especially if non-obese and hypertensive.

PO6-10
A randomized controlled trial of Resmetirom in non-alcoholic steatohepatitis: 52-week Data From MAESTRO-NASH

Jörn Schattenberg[1], Stephen Harrison[2], Pierre Bedossa[3], Cynthia Guy[4], Rohit Loomba[5], Rebecca Taub[6], Dominic Labriola[6], Sam Moussa[7], Guy Neff[8], Arun Sanyal[9], Mazen Noureddin[10], Meena Bansal[11], Naim Alkhouri[12], Vlad Ratziu[13]
[1]University Mainz, Mainz, Germany, *[2]Pinnacle Clinical Research, San Antonio, United States*, *[3]Newcastle University, Newcastle, United Kingdom*, *[4]Duke University, Raleigh, United States*, *[5]UC San Diego, San Diego, United States*, *[6]Madrigal Pharmaceuticals, Conshohocken, United States*, *[7]University of Arizona, Tuscon, United States*, *[8]Covenant Research, Sarasota, United States*, *[9]Virginia Commonwealth University, Richmond, United States*, *[10]Houston Methodist Hospital, Houston, United States*, *[11]Icahn School of Medicine at Mt Sinai, New York, United States*, *[12]Arizona Liver Institute, Chandler, United States*, *[13]Pitie-Salpetriere Hospital, Sorbonne Universite, Paris, France*
Email: joern.schattenberg@unimedizin-mainz.de

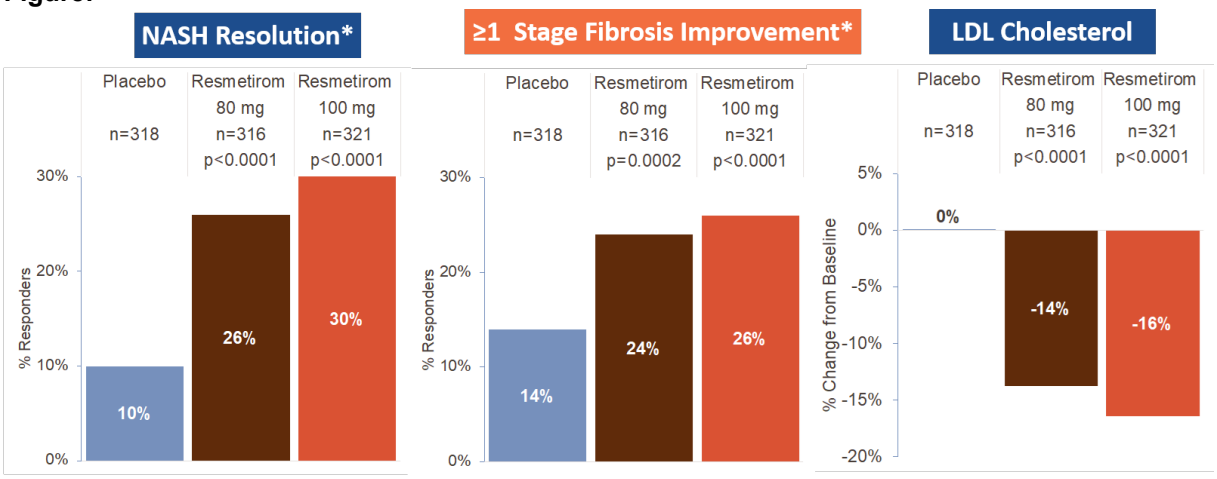
Background and aims: MAESTRO-NASH (NCT03900429) is an ongoing 54-month, Phase 3, registrational double blind, placebo-controlled NASH clinical trial to study the effect of once daily 80 mg or 100 mg resmetirom versus placebo in patients with NASH and liver fibrosis.

Method: 966 patients were enrolled at ~200 sites. Eligibility required: presence of >3 metabolic risk factors, vibration-controlled transient elastography (VCTE) ≥8.5 kPa, MRI-PDFF ≥8% and biopsy-proven NASH with fibrosis stage 1B, 2, or 3 and NAFLD activity score (NAS) ≥4. Week 52 dual primary end points included resolution of NASH (ballooning 0, inflammation 0, 1 with ≥2-pt reduction in NAS) with no worsening of fibrosis OR ≥1 stage reduction in fibrosis with no worsening of NAS. The key secondary end point was % reduction in LDL-C at Week 24. Biopsies were read by two central pathologists using glass slides (primary analysis); results combined using a statistical algorithm to generate a single treatment effect. A supportive consensus read using digitized images was conducted if readers disagreed on either primary end point. The mITT population excluded 11 patients with liver biopsies after Week 60 due to COVID issues.

Results: Baseline characteristics: age 57 (11) (mean (SD)), female 56%, white 90%, BMI 36 (7), type 2 diabetes 67%, hypertension 78%, dyslipidemia 71%, VCTE 13 kPa (7), CAP 348 (38), MRI-PDFF 18% (7) fat fraction, baseline liver biopsy NAS ≥5 84%, baseline fibrosis stage: F3-62%, F2-33%, F1B-5%. Both primary histologic end points and the key secondary end point were met (table) as were other biopsy end points. Similar results for both end points were obtained by both central pathologists. Biomarker end points including reductions in liver enzymes, ELF, MRI-PDFF, CAP and VCTE were reduced. There were similar numbers of SAEs across groups. Observed increase in the incidence of diarrhea and nausea in resmetirom treatment groups at the beginning of therapy.

Conclusion: NASH resolution and fibrosis reduction end points on liver biopsy were achieved at both doses of resmetirom in a large Phase 3 pivotal clinical trial. Resmetirom appeared safe and was generally well-tolerated. These data support the potential for resmetirom treatment to provide benefit to patients with NASH and liver fibrosis.

Figure:


NASH Resolution*
Placebo
n=318
Resmetirom
80 mg
n=316
p<0.0001
Resmetirom
100 mg
n=321
p<0.0001
% Responders
30%
20%
10%
0%
10%
26%
30%
≥1 Stage Fibrosis Improvement*
Placebo
n=318
Resmetirom
80 mg
n=316
p=0.0002
Resmetirom
100 mg
n=321
p<0.0001
% Responders
30%
20%
10%
0%
14%
24%
26%
LDL Cholesterol
Placebo
n=318
Resmetirom
80 mg
n=316
p<0.0001
Resmetirom
100 mg
n=321
p<0.0001
% Change from Baseline
5%
0%
-5%
-10%
-15%
-20%
0%
-14%
-16%

PO6-11-YI
The beneficial hepatic-effect of Glucagon-like Peptide 1 receptor agonists in diabetic patients with metabolic dysfunction-associated liver disease (MASLD)

Huma Malik[1], Roberta Forlano[1], Giordano Sigon[1], Vicky Lin[1], Jian Huang[1], Thishana Kirupakaran[1], Margaux Couffon[1], Maryam Shapoor[1], Benjamin H. Mullish[1], Mark Thursz[1], Michael Yee[2], Pinelopi Manousou[1]
[1]*Imperial college London, Department of Metabolism, Digestion and Reproduction*, [2]*Imperial college London, Department of Endocrinology*
Email: r.forlano@imperial.ac.uk

Background and aims: Metabolic dysfunction-associated liver disease (MASLD) is the most prevalent chronic liver disease worldwide. As there is no licensed pharmacotherapy, weight loss and lifestyle remain the mainstay for treating the condition. Glucagon-like peptide 1 receptor agonists (GLP-1Ras) have shown promise in MASLD treatment, as they promote significant weight loss. In this study, we assessed the effect of long-term GLP-1 therapy on liver disease severity in a *real-life* cohort of patients with MASLD.

Method: This retrospective observational study included all new NAFLD patients seen in the specialist liver clinic (Imperial college Healthcare NHS Trust) from January 2010 to May 2022. Demographic, anthropometric, and clinical data was collected at baseline, before the initiation of GLP1Ra and at their most recent follow-up. Liver stiffness measurement (LSM) and Controlled attenuation parameter (CAP) score were recorded during the clinic visits.

Results: Overall, 779 patients were included. Among those with type 2 diabetes mellitus (T2DM) (335/779 = 43%), 94 (28%) were prescribed a GLP-1RA for a median period of 38 (1-171.2) months. At the end of follow-up, there was a significant improvement in weight (95 vs 92 kg, p = 0.005), ALT levels (46 vs 38 IU/L, p = 0.003) and HbA1c (63 vs 58 mmol/l, p = 0.009) compared to baseline in those taking GLP-1RA. In a matched analysis per age and gender, patients on GLP-1RA showed greater weight loss (delta BMI -1.32 vs -0.32 kg/m2, p = 0.037) and reduction in CAP score (delta CAP score -32 vs -4 dB/m, p = 0.024) over the follow-up compared to those who were not taking the medication. A greater reduction in CAP score remained significant also in the subgroup of patients who did not lose weight (delta CAP score -32 vs -10 dB/m, p = 0.036). When compared to diabetics on other anti-diabetic medications, patients on GLP-1RA showed the greatest decrease in BMI (delta BMI 0.88 kg/m2) and in CAP score (delta CAP score 23 dB/m). There was no difference in terms of FIB-4 or LSM. There were no differences in terms of clinical outcomes, such as major adverse cardiovascular events, decompensated events, or all-cause mortality.

Conclusion: In this real-life cohort of diabetic patients with MASLD, treatment with GLP-1RA was associated with greater weight loss and hepatic fat reduction. Of note, a reduction in fat content was observed also in those who did not lose weight. Longer studies are required to assess the effect of GLP-1RA therapy on fibrosis changes. Based on these results, GLP-1RA therapy should be favoured when treating diabetic patients with MASLD.

PO6-12
Association between FAST™ score and hepatocyte ballooning in metabolic dysfunction-associated steatohepatitis patients: findings from EVIDENCES-X study of 393 biopsied individuals

Taufik Momin[1], Deven Parmar[2], Arun Sanyal[3], Naga Chalasani[4], Nehal Parikh[1]
[1]Zydus Lifesciences Ltd, *[2]Zydus Therapeutics Inc*, *[3]Virginia Commonwealth University*, *[4]Indiana University School of Medicine*
Email: dparmar@zydustherapeutics.com

Background and aims: Liver biopsy is the gold standard diagnostic and assessment procedure in Metabolic dysfunction-associated steatohepatitis (MASH) clinical trials. However, meeting the histologic criteria is quite a challenging factor with the high screen failure rate in the MASH clinical trials. Non-invasive tests (NITs) have an important role in identifying at-risk MASH patients having advanced fibrosis. The presence of hepatocytic ballooning is the most challenging histologic feature to predict from NITs. We evaluated the correlation of NITs with hepatocyte ballooning in a large cohort of patients with liver histology.

Method: We used data from the ongoing EVIDENCES-X Saroglitazar Phase 2b trial *[NCT05011305]*. We analyzed demographics, laboratories, Fibroscan®, and liver histology reading by a central pathologist in 393 patients with well-characterized MASH participants across the United States and Latin America. We evaluated the correlation of NITs with the presence of hepatocyte ballooning in the biopsy. Descriptive statistics were calculated for participants with and without hepatocyte ballooning. The receiver operating characteristic (ROC) curve was used for the Area under the ROC (AUROC) comparison and cut-off point selection. Youden's J index method was used for deciding an optimum cut-off for Fibroscan-AST (FAST) score.

Results: Out of 790 screened patients, 393 liver biopsies were performed and included in this analysis. 117 (30%) were qualified for enrollment as per the eligibility criteria for MASH (NAS ≥4 and F23). Ballooning was not detected in 202 of 393 centrally read biopsies (51%). Patients with hepatocyte ballooning had higher liver enzymes, glycated hemoglobin (HbA1c), and Liver stiffness measurement (LSM) values (see Table). NITs like aspartate aminotransferase to platelet ratio index (APRI), Fibrosis-4 (FIB-4), FAST, agile 3+, and agile 4 were higher in patients having hepatocyte ballooning. FAST (Fibroscan-based) and APRI (blood-based) outperformed other NITs in terms of AUROC. In subjects with ballooning grade 0, 1 and 2; FAST score (Mean [95%CI]) was 0.37 [0.34-0.40], 0.53[0.49-0.57], and 0.63[0.58-0.67], respectively (see Figure). FAST score threshold of 0.41 correctly identified ballooning in a total of 260 (70%) out of 369 patients, with a sensitivity of 61%, specificity of 80%, NPV of 80%, and PPV of 76%. FAST cutoff 0.41 predicts accurately at-risk NASH (NAS≥4 and F≥2) in 85% of patients and rules out early disease (NAS<4 and F<2) in 70% of patients.

Conclusion: NITs provide valuable insights into identifying target MASH patients having hepatocyte ballooning and help reduce screening failure due to biopsy. A FAST score cutoff of 0.41 has good predictability to detect hepatocytic ballooning

Figure: Correlation of FAST score with Ballooning grade

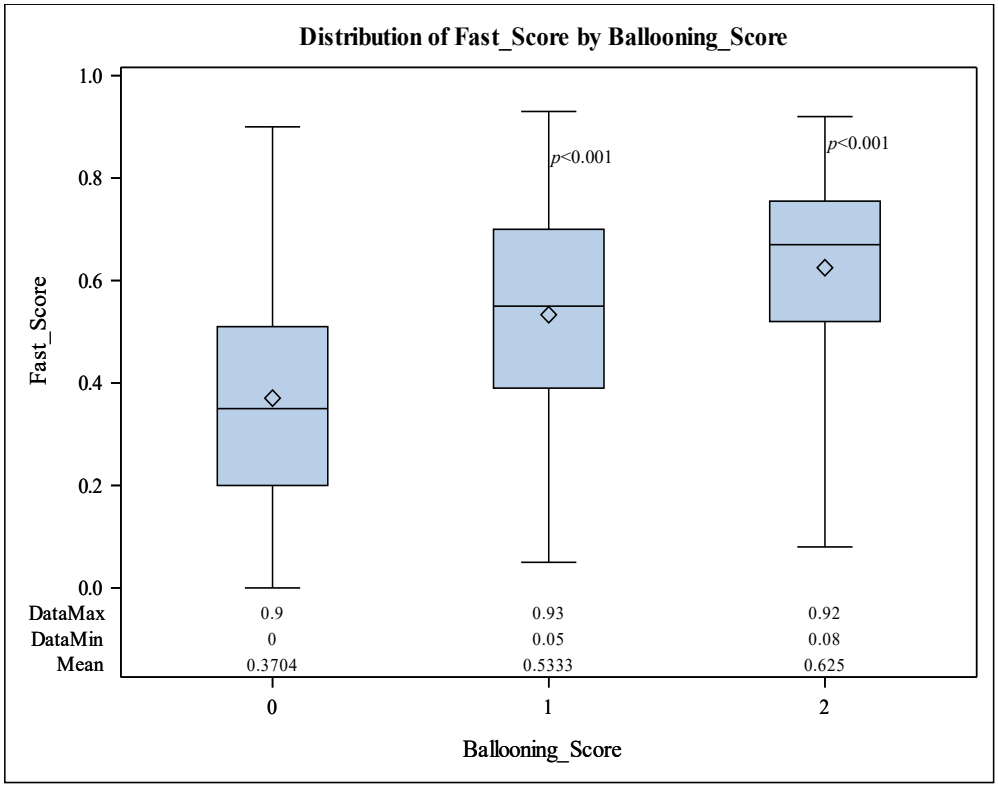


Table: Variables Affecting Ballooning

	No ballooning (n = 202)	Ballooning (n = 191)	p value
	Mean (SD)		
Age (year)	54 (12)	55 (11)	0.39
BMI (kg/m2)	35.8 (5.2)	34.9 (5.5)	0.10
AST, IU/L	30.9 (19.2)	52.6 (28)	**<0.0001**
ALT, IU/L	42.3 (31.9)	65.2 (39.4)	**<0.0001**
GGT, IU/L	50.2 (59.3)	69.7 (60)	<0.01
Platelet	263 (66.4)	244.7 (65.9)	<0.01
Triglyceride, mg/dl	158.2 (71.8)	165.1 (77.7)	0.36
HbA1c	6.1 (0.9)	6.5 (1)	**<0.0001**
NFS	-1.4 (1.3)	-0.9 (1.3)	**0.0002**
APRI	0.4 (0.2)	0.7 (0.4)	**<0.0001**
FIB-4	1.07 (0.49)	1.59 (0.73)	**<0.0001**
FAST	0.37 (0.22)	0.57 (0.20)	**<0.0001**
Agile 3+	0.39 (0.26)	0.55 (0.26)	**<0.0001**
Fibroscan CAP	333.1 (45)	328.4 (43.2)	0.30
Fibroscan LSM	10.1 (5.3)	11.6 (5)	<0.01

PO6-13-YI
Estimated glucose disposal rate correlates with histological severity of MASLD in women, but not in men

Jonathan Mertens[1 2], Zouhir Gadi[3], Wilhelmus Kwanten[2 3], An Verrijken[1 2], Kristof Van Dessel[1 2], Eveline Dirinck[1 2], Luisa Vonghia[2 3], Christophe De Block[1 2], Sven Francque[2 3]
[1]Antwerp University Hospital, Endocrinology, Diabetology and Metabolism, Edegem, *[2]University of Antwerp, Laboratory of Experimental Medicine and Paediatrics, Wilrijk*, *[3]Antwerp University Hospital, Gastroenterology and Hepatology, Edegem*
Email: jonathan.mertens@student.uantwerpen.be

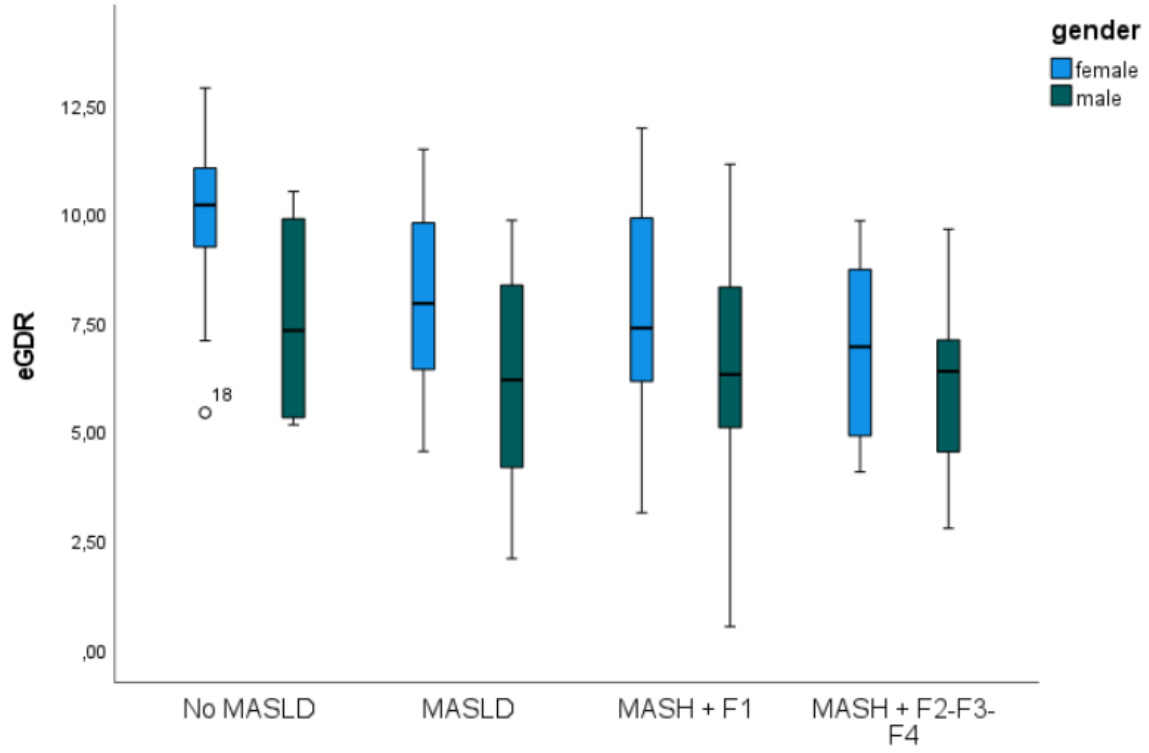
Background and aims: Metabolic Dysfunction Associated Steatotic Liver Disease (MASLD) is closely linked to insulin resistance (IR). Previous studies have demonstrated a correlation between liver histology and the homeostatic model assessment for IR (HOMA-IR). The estimated glucose disposal rate (eGDR) is a clinically derived index of IR, utilizing readily available clinical parameters. This study aimed to investigate whether eGDR correlates with histological severity in MASLD.

Method: A retrospective analysis was conducted on 258 liver biopsies obtained from individuals with clinical suspicion of MASLD between 2006 and 2019. Subjects with alcohol intake >2 units/d in women and >3 units/d in men were excluded. Histology ranged from normal livers without steatosis over isolated steatosis to Metabolic Dysfunction Associated Steatohepatitis (MASH) with/without significant fibrosis. eGDR was calculated using the formula proposed by Williams et al. (2000), based on waist-to-hip ratio, hypertension, and HbA1c levels. Based on histology patients were categorized into no MASLD (no steatosis), MASL (steatosis only), and MASH without or with significant fibrosis (F0-F1 vs. F2-F4), or dichotomized into no MASLD vs. MASLD/MASH. Gender-stratified analyses were conducted to investigate potential sex-specific correlations.

Results: Cohort included 170 women, and 88 men. Median eGDR was 5.74 mg/kg/min [7.60-9.70]. People with diabetes had significantly lower eGDR (4.82 [3.83-6.32] vs. 7.82 [5.99-9.81], p <0.001). eGDR also differed significantly according to sex (6.25 [4.88-8.29] in males vs. 8.27 [6.40-10.10] in females, p <0.001). There was a statistically significant difference in eGDR across histological stages (p <0.001). Pairwise post-hoc comparison with Bonferroni correction for multiple testing revealed a significant difference in eGDR value between no MASLD and MASH with and without advanced fibrosis, and between no MASLD and MASLD. When looking at individual components of histology, eGDR was significantly higher in S0 versus S1, S2 and S3 (p <0.001), higher in those without ballooning compared to both moderate or severe ballooning (p <0.001) and higher in F0 compared to F2, but not compared to other fibrosis stages. When stratified by sex, these differences appeared to be restricted to women but not men, with the exception of ballooning, which had a significantly different distribution of eGDR between no ballooning and moderate/severe ballooning also in men. When dichotomizing histology in no MASLD versus MASLD/MASH, only in females eGDR was significantly higher in those without MASLD (p <0.001). Univariable logistic regression showed that eGDR was associated with MASLD/MASH in females (OR: 0.53 [0.40-0.69], p <0.001) but not in males. Multivariable logistic regression only showed an association between eGDR and MASLD/MASH in females (OR: 0.56 [0.39-0.81], p = 0.002), while waist circumference, age, diabetes, HbA1c, and systolic/diastolic blood pressure did not, while this could not be observed in men.

Conclusion: In a cohort of 258 individuals, eGDR correlated with the histological presence and severity of MASLD/MASH in women, but not in men.

Figure:


gender
female
male
eGDR
12,50
10,00
7,50
5,00
2,50
,00
18
No MASLD
MASLD
MASH + F1
MASH + F2-F3-F4

PO6-14
Phosphatilylethanol probe into the de-novo post-liver transplant steatotic liver disease: MASLD, or MetALD?

Lubomir Skladany[1], Svetlana Adamcova Selcanova[1], Natalia Bystrianska[1], Daniela Žilinčanová[1], Karolina Sulejova[1], Daniel Jan Havaj[1], Miroslav Vybostok[2], Peter Jarcuska[3]
[1]F. D. Roosevelt University Hospital in Banská Bystrica, Dept Internal Med II, Div. Hepatology, Gastroenterology and Liver transplantation, Banska Bystrica, Slovakia, *[2]Department of Mathematics, Faculty of Natural Sciences, Matej Bel University, Banska Bystrica*, *[3]Košice Medical University, Dept Internal Med II, Kosice, Slovakia*
Email: lubomir.skladany@gmail.com

Background and aims: According to the Global Burden of Diseases study, Slovakia has the highest prevalence of liver cirrhosis in the world with alcohol-associated liver disease (ALD) being the most prevalent, and metabolic-associated fatty liver disease the fastest growing etiology, respectively (MetSLD). As both ALD and MetSLD pathogenetic cascades are operative post-liver transplant (LT), we aimed to decipher their relative contribution to post-LT SLD in this study.

Method: Between July 2020 and April 2023, we carried out a prospective study on consecutive patients, transplanted for indications other than SLD. We recorded baseline (pre-LT) demographics, cirrhosis etiology, and MELD score. For the post-LT contribution of SLD, we assessed (at months 6-12-24) magnetic resonance spectrography for liver fat content (MRS, [≥5% = de novo post-LT NAFLD], and elastography for fibrosis (MRE [≥2.88kPa = significant fibrosis, ≥3.54kPa = advanced fibrosis]). For the contribution of ALD on post-LT STD, we evaluated alcohol relapse at 3m intervals using phosphatidyl ethanol (PEth; umol/l).

Results: We included 90 patients and analyzed 75 patients, respectively, with a mean age of 50.94 years (15-70), MELD 16, 08 points. The most prevalent indications for LT were ALD (56%), and autoimmune syndromes (29%). De novo NAFLD at 6-12-24 months post-LT was detected in 18%-25%-and 22% of patients. Overall, alcohol slips/relapse was diagnosed in 10.7% of patients. The mean overall PEth (ULN <0.02) was 0.037 umol/l (0.02-0.51). In patients with/wo de-novo NAFD, PEth levels at 6-12-and 24 months were 0.033 *vs*. 0.057 (p = 0.53), 0.043 *vs*. 0.025 (p = 0.12), and 0.046 *vs*. 0.02 (p = 0.11), respectively. In patients with/wo significant fibrosis (MRE≥2.88), the PEth at 6-12-and 24 months were 0.02 *vs*. 0.034 (p = 0.25), 0.025 *vs*. 0.030 (p = 0.67), and 0.02 *vs*. 0.055, (p = 0.33), respectively.

Conclusion: In our cohort, de-novo NAFLD mostly represents pure MetSLD; the question is if the post-LT MetSLD is an entity exceeding the framework of MetSLD to the extent of special designation.

PO6-15-YI
Diagnostic accuracy and optimal cut-off of fibroScan controlled attenuation parameter (CAP) and liver stiffness measurement (LSM) in assessing steatosis and fibrosis in patients with bariatric surgery and elective laparoscopic cholecystectomy

Riham Soliman[1 2], Mohammed Elbasiony[1 3], Mohammed Emam[1], Ayman Hassan[1], Nabiel Mikhail[1], Tarek Salah[1 3], Helmy Ezzat[1 3], Gamal Shiha[1 3]
[1]Egyptian Liver research Institute and Hospital, Egypt, *[2]Faculty of Medicine, Port Said University*, *[3]Faculty of Medicine, Mansoura University*
Email: rehamelsayed@hotmail.com

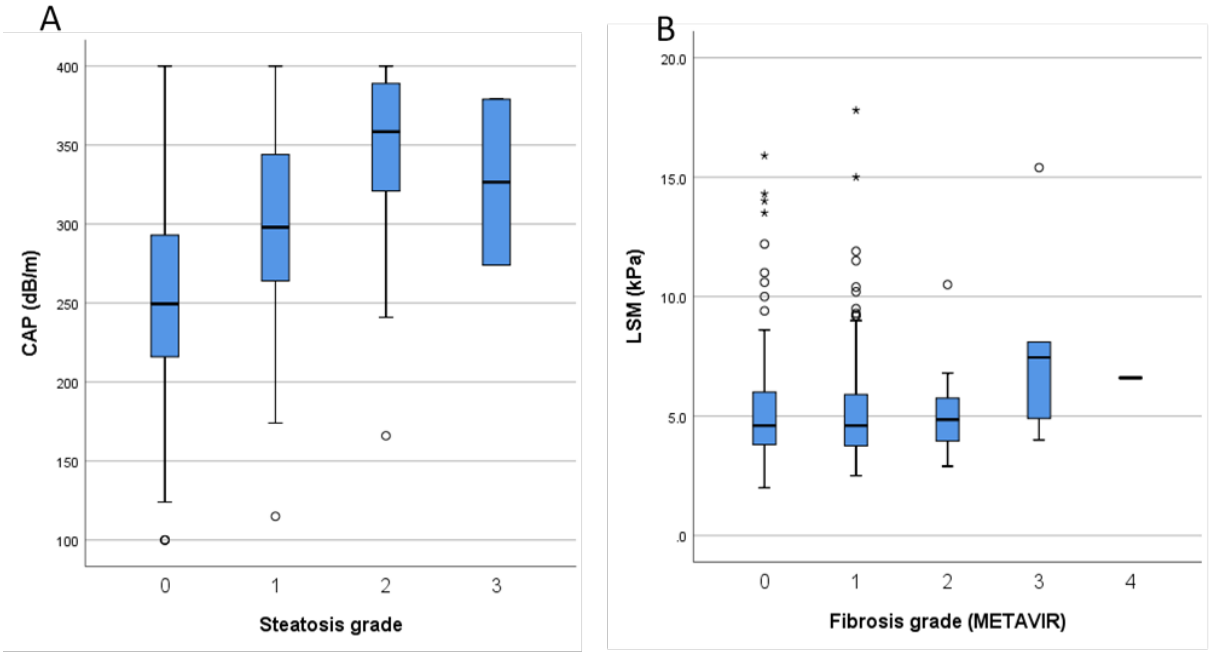
Background and aims: Evaluating liver steatosis and fibrosis is important for patients with non-alcoholic fatty liver disease. Although liver biopsy and pathological assessment is the gold standard for these conditions, this technique has several disadvantages. We evaluated the performance of CAP and LSM for assessing steatosis and fibrosis in patients with bariatric surgery and elective laparsopic cholecystectomy in comparison to liver biopsy.

Method: This is a cross-sectional prospective single center study including 297 patients who had laparoscopic cholecystectomy or bariatric surgery at the Egyptian Liver Research Institute and Hospital (ELRIAH) between May 2019 and May 2023. Results of CAP and LSM were compared with the Liver histology. Experienced pathologists using METAVIR scoring system scored the biopsy samples. Overall diagnostic accuracy of CAP and LSM was estimated as the AUROC together with its 95% CI. Cutoff values for CAP and LSM were identified that maximize the Youden index

Results: According to histological assessment, steatosis grade distribution was as follows: S0 = 193 (65%), S1 = 78 (26.3%), S2 = 23 (7.7%), S3 = 3 (1%)/Accuracy at the S≥S1 threshold was with an AUROC of 0.76 (95% CI 0.701-0.819) and sensitivity of 0.693 (0.593-0.781) and specificity of 0.726 (0.657-0.788) at a threshold of 290 dB/m selected by maximizing Youden's index. Accuracy increased to an area under the curve (AUC) of 0.841 (0.749-0.932) for the S≥S2 threshold, with the corresponding sensitivity of 0.833 (0.626-0.953) and specificity of 0.809 (0.757-0.854) at the threshold of 317 dB/m maximizing Youden's index.Similar results were obtained for LSM with a cut off value of 4.7 and 6, 6 KPa for ≥F2 and ≥F3 respectively

Conclusion: These new cutoff values identified for CAP and LSM effectively discriminate between different grades of steatosis and fibrosis, enabling clinicians to accurately diagnose and monitor non-alcoholic fatty liver disease.

Figure:


A
CAP (dB/m)
400
350
300
250
200
150
100
0
1
2
3
Steatosis grade
B
LSM (kPa)
20.0
15.0
10.0
5.0
.0
0
1
2
3
4
Fibrosis grade (METAVIR)

PO6-16
Steatotic liver disease and inflammatory bowel disease: is it all MASLD?

Patrícia Sousa[1], Pedro Monsanto[1], Luís Silva[2], Patrícia Nicolau[1], Andreia Nascimento[1], Beatriz Bartissol[3], Gisela Rocheta[3], Susana Rodrigues[3], Sara Alberto[1], Paula Cunha[1], Rosário Maldonado[1], Mariana Machado[1 4]
[1]Hospital de Vila Franca de Xira, Gastroenterology, Lisbon, Portugal, *[2]Hospital de Vila Franca de Xira, Clinical Pathology, Lisbon, Portugal*, *[3]Hospital de Vila Franca de Xira, Nutrition, Lisbon, Portugal*, *[4]Faculdade de Medicina, Lisbon University, Gastroenterology and Hepatology, Lisbon, Portugal*
Email: mverdelhomachado@gmail.com

Background and aims: Steatotic liver disease (SLD) seems prevalent in inflammatory bowel disease (IBD) patients, albeit IBD patients less frequently present metabolic dysfunction compared to other SLD high-risk populations. We aimed to evaluate the prevalence of metabolic-associated SLD (MASLD), and compare it with non-MASLD SLD, in IBD patients.

Method: Prospective study evaluating IBD outpatients, with assessment of liver steatosis and fibrosis with Fibroscan®. A food frequency questionnaire (FFQ) allowing the quantification of energy and 44 nutrients and a Mediterranean diet (MD) adherence score (PREDIMED) were applied. Body composition was assessed with bioelectrical impedance analysis and PA with International Physical Activity Questionnaire.

Results: 115 patients were included, 42% male, age 46 ± 14 years. 44% had Crohn's disease and 56% ulcerative colitis. 48% (55 patients) presented SLD (CAP > 248dB/m) and 11% significant fibrosis (elastography>8kPa). Only 4 patients with SLD (7%) did not fulfill criteria for MASLD. Compared to MASLD patients, non-MASLD SLD patients were younger (43 ± 2 vs. 51 ± 2 years, P = 0.029), with lower visceral fat (visceral fat rate 3 ± 2 vs. 12 ± 1, P = 0.014), lower aminotransferases and GGT levels (AST 13 ± 2 vs. 23 ± 1, P = 0.031; ALT 24 ± 3 vs. 42 ± 3, P = 0.003; GGT 25 ± 6 vs. 47 ± 6, P = 0.046), lower inflammatory markers (ESR 2 ± 2 vs. 11 ± 2 mm/h, P = 0.020; ferritin 8 ± 4 vs. 155 ± 24 ng/ml, P = 0.001) and lower hepatic elastography (4.5 ± 0.5 vs. 6.3 ± 0.4 kPa, P = 0.035) or FAST score (0.12 ± 0.01 vs. 0.19 ± 0.02, P = 0.0001). There were no differences regarding physical activity or diet, including adherence to MD, except for lower consumption of soft drinks (0.75 ± 0.25 vs. 3.38 ± 0.87 units/week, P = 0.005), milk (0.125 ± 0.125 vs. 3.01 ± 0.655 units/week, P = 0.001), and alcohol (0.07 ± 0.07 vs. 3.38 ± 0.87 units/day, P = 0.005).

Conclusion: SLD is highly prevalent in patients with IBD, in whom, 9 out of 10 patients with SLD present MASLD. Non-MASLD SLD, in IBD, seems to be a less severe disease, and may translate a precursor form of MASLD or a different entity.

PO6-17
Effects of sodium-glucose cotransporter 2 inhibitor versus sulfonylurea on non-alcoholic fatty liver disease and diabetes

Yumie Takeshita[1], Masao Honda[2], Shuichi Kaneko[2], Toshinari Takamura[3]
[1]Kanazawa University Graduate School of Medical Sciences, Department of Endocrinology and Metabolism, kanazawa, Japan, *[2]Kanazawa University Graduate School of Medical Sciences, Department of Gastroenterology, Kanazawa, Japan*, *[3]Kanazawa University Graduate School of Medical Sciences, Department of Endocrinology and Metabolism, Kanazawa, Japan*
Email: takeshita@m-kanazawa.jp

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a liver phenotype of type 2 diabetes and obesity. The efficacy of sodium-glucose cotransporter 2 (SGLT2) inhibitors and sulfonylureas in liver pathology and hepatic gene expression profiles 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 liver histology. The secondary end points were the changes in liver enzymes, metabolic markers, and hepatic gene expression profiles.

Results: Fibrosis scores improved in the tofogliflozin group (60%), but 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. The tofogliflozin group significantly reduced weight, BMI, and body fat percentage. Genes involved in gluconeogenesis, fatty acid catabolism/oxidation, and amino acids catabolism in peroxisome were coordinately upregulated in the tofogliflozin group. On the other hand, genes involved in cell death, stress response, inflammation, T-cell response, and fibrosis were substantially downregulated in the tofogliflozin group but not in the glimepiride group.

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. The hepatic expression of the genes involved in energy metabolism, inflammation, and fibrosis was well correlated with liver histological changes and rescued by tofogliflozin.

Figure: Differential signalling pathways in the liver of NAFLD participants altered by treatment with tofogliflozin or glimepiride

No.	KEGG pathway	Pathway description	Number of genes	LS permutation p-value	KS permutation p-value	Up or down	Representative genes
Tofogliflozin							
	Metabolism						
1	hsa00071	Fatty acid degradation	43	0.00001	0.00001	Up	ACSL5, GCDH, ACADSB
2	hsa00250,hsa00260, hsa00280, hsa00340, hsa00380	Amino acids metabolism (Ala, Asn, Gln, Gly, Ser, Thr, Val, Leu, Ile, His, Trp)	172	0.00001	0.00001	Up	AGXT, GNMT, BCKDHB
3	hsa00980	Metabolism of xenobiotics by cytochrome P450	64	0.00001	0.00009	Up	GSTA2, ALDH3A1, UGT1A7
4	hsa03320	PPAR signaling pathway	64	0.00001	0.00001	Up	SLC27A5, APOA5, ACOX2
5	hsa04146	Peroxisome	79	0.00001	0.00001	Up	PXMP2, PHYH, MLYCD
6	hsa00051	Fructose and mannose metabolism	35	0.00005	0.00079	Up	PMM1, ALDOB, FBP1
7	hsa00830	Retinol metabolism	59	0.00020	0.00022	Up	CYP2A6, CYP2A7, CYP1A1
8	hsa00010	Glycolysis / Gluconeogenesis	58	0.00047	0.00363	Up	PCK1, ENO3, G6PC
9	hsa00020	Citrate cycle (TCA cycle)	29	0.00374	0.06582	Up	PCK2, ACO1, SDHB
10	hsa00190	Oxidative phosphorylation	97	0.00891	0.00175	Up	COX6C, COX17, NDUFB7
	Cell cycle						
1	hsa03030	DNA replication	36	0.00001	0.0026	Down	MCM2, MCM6, PRIM2
2	hsa04110	Cell cycle	124	0.00001	0.00001	Down	CDC7, CHEK1, CCNB1
	Apotosis/inflammation						
1	hsa04612	Antigen processing and presentation	62	0.00015	0.00165	Down	TAP1, HLA-DQB1, CIITA
2	hsa05340	Primary immunodeficiency	32	0.00042	0.00183	Down	CD3E, JAK3, IL2RG
3	hsa04210	Apoptosis	84	0.00256	0.00223	Down	BIRC3, TNFRSF10D, FAS
4	hsa04010	MAPK signaling pathway	229	0.00349	0.00942	Down	PDGFRB, CD14, FGF2
5	hsa04670	Leukocyte transendothelial migration	105	0.01046	0.00038	Down	CXCR4, CLDN7, ICAM1
	Fibrosis						
1	hsa04510	Focal adhesion	190	0.00001	0.00001	Down	COL4A1, COL1A1, LAMA3
2	hsa04512	ECM-receptor interaction	80	0.00001	0.00057	Down	HMMR, ITGA9, PDGFRA
3	hsa04514	Cell adhesion molecules (CAMs)	122	0.00002	0.00009	Down	CNTNAP2, SDC2, CLDN7
Glimepiride							
	Metabolism						
1	hsa04146	Peroxisome	79	0.0017	0.00001	Up	PEX7, HACL1, GNPAT
2	hsa00071	Fatty acid degradation	43	0.0085	0.00048	Up	ADH7, ALDH3A2, ADH1A
3	hsa00980	Metabolism of xenobiotics by cytochrome P450	64	0.0271	0.00445	Up	UGT2B15, UGT1A1, UGT2B10

PO6-18
Lipid and carbohydrate metabolism in children with metabolic-associated fatty liver fibrosis

Nataliya Zavhorodnia[1], Inna Klenina[2], Oksana Tatatrchuk[2], Olena Hrabovskaya[2]
[1]SI "Institute Gastroenterology of the National Academy of Medical Sciences of Ukraine, Pediatric Gastroenterology Department, Dnipro, Ukraine, *[2]SI "Institute Gastroenterology of the National Academy of Medical Sciences of Ukraine, Scientific Research Department, Dnipro, Ukraine*
Email: nzavgorodni75@gmail.com

Background and aims: Metabolic-associated fatty liver disease known as the hepatic manifestation of metabolic syndrome is the most common type of chronic liver disease worldwide. Chronic hepatic inflammation promoted by metabolic dysfunction can cause changes in liver structure and function, potentially leading to organ failure and cirrhosis. Therefore, the aim of our study was to investigate the parameters of lipid and carbohydrate metabolism in children with metabolic-associated fatty liver fibrosis.

Method: 80 obese children were included in the study. According to transient elastography data (Fibroscan502touch, Echosense, France), obese patients were divided into 3 groups: 1 group consisted of 27 MAFLD children with fibrosis ≥F1, 2 group-35 MAFLD children without fibrosis, 3 group- 18 obese children without MAFLD and fibrosis. The control group (4 group) consisted of 14 children with normal weight without MAFLD and fibrosis. Serum total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) were determined with Cormey reagent kits (Poland) using a Stat Fax 1904 Plus analyzer (Awareness Technology, USA). Low-density lipoproteins (LDL) were calculated using the Friedwald formula: LDL = TC-HDL-TG/2.18 (mmol/L). Very low-density lipoproteins (VLDL), the atherogenicity coefficient (AC) were calculated. The serum insulin content was evaluated by an enzyme-linked immunosorbent assay using a test kit from DRG International, Inc. (Germany). HOMA-IR was calculated by the formula: HOMA-IR = fasting glucose (mmol/L) x fasting insulin (μU/ml)/22.5.

Results: Median TG levels were increased in 1 group in 1.7 times ($p < 0.001$), VLDL-in 1.7 times ($p < 0.001$) compared to 4 group patients. Significant differences in TG content were found between 1 group patients and 2 group ($p = 0.031$) and 3 group ($p = 0.025$). A decrease in HDL levels was detected in all group patients: in 1 and 2 group by 1.3 times ($p < 0.001$), in 3 group-by 1.2 times ($p < 0.05$) compared to 4 group. Lipid fractions imbalance led to an increase in AC level: in 1 and 3 groups by 1.5 times ($p < 0.05$), in 2 group-by 1.4 times ($p < 0.01$).
Significant differences in carbohydrate metabolism were demonstrated. In 1 group patients an increase in the median insulin level by 2.3 times ($p < 0.05$) and HOMA-IR level by 2.3 times ($p < 0.05$) was found compared to the control group. It should be noted that in 1 group children an increase in median insulin level by 1.4 times ($p > 0.05$) and HOMA-IR by 1.6 times ($p > 0.05$) was observed compared to 3 group patients.

Conclusion: Thus, in children with metabolic-associated fatty liver fibrosis lipid abnormalities such as an increase in serum TG, LDL, AC levels, and a decrease in HDL levels as well as insulin resistance were observed which may contribute to liver fibrosis progression in children with MAFLD.

PO7-01-YI

Association between cardiovascular disease risk factors and metabolic dysfunction-associated steatotic liver disease among rheumatoid arthritis patients

Alina Saidi[1], Willy Theel[2], Vivianne de Jong[3], Benjamin Burggraaf[4], Aart-Jan van der Lelij[4], Diederick Grobbee[5], Ellen van der Zwan-van Beek[6], Manuel Castro Cabezas[1]
[1]Franciscus Gasthuis and Vlietland, Department of Internal Medicine and Endocrinology, Rotterdam, Netherlands, *[2]Franciscus Gasthuis and Vlietland, Department of Internal Medicine, Rotterdam, Netherlands*, *[3]Julius Clinical, Department of Science, Zeist, Netherlands*, *[4]Erasmus University Medical Center, Department of Internal Medicine, Rotterdam, Netherlands*, *[5]Julius Clinical, Department of Science, Zeist, Netherlands*, *[6]Franciscus Gasthuis and Vlietland, Department of Clinical Chemistry, Rotterdam, Netherlands*
Email: a.saidi@franciscus.nl

Background and aims: Rheumatoid arthritis (RA) is associated with an increased risk for atherosclerosis and with metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD). The effect of MASLD on the cardiovascular risk among RA patients is not well known. The Franciscus Rheumatoid Arthritis and Cardiovascular Intervention Study (FRANCIS) study aims to evaluate cardiovascular risk in patients with RA. FRANCIS participants with different risks of liver fibrosis were evaluated for the presence of subclinical atherosclerosis.

Method: The association between hepatic fibrosis, using the fibrosis-4 (FIB-4) index and traditional cardiovascular disease (CVD) risk factors with subclinical atherosclerosis assessed as carotid Intima Media Thickness (cIMT) by ultrasound among RA patients aged <70 years without clinical CVD and without diabetes type 2 (T2DM) was evaluated in the FRANCIS study.

Results: In total, 317 RA patients were included. The mean age (± SD) of the population was 54 (± 11) years and 215 patients (68 %) were female. Compared to patients with low FIB-4 (≤1.3) RA patients in the elevated FIB-4 group (>1.3) had higher cIMT values (0.62 ± 0.12 mm vs. 0.57 ± 0.11 mm), higher apolipoprotein (apo) B48 concentrations (12.0 ± 7.1 mg/L vs. 9.7 ± 6.6 mg/L, higher systolic blood pressure (139 ± 23 mm Hg vs. 131 ± 18.6 mmHg) ($p = 0.002$, $p = 0.029$, and $p = 0.007$ respectively). Systemic inflammatory markers like leukocytes, CRP and complement component 3 were all significantly lower in RA subjects with elevated FIB-4. FIB-4 correlated positively with cIMT ($r = 0.319$; $p < 0.001$), systolic blood pressure ($r = 0.254$; $p < 0.001$), total cholesterol ($r = 0.146$; $p = 0.009$), LDL-C ($r = 0.127$; $p = 0.026$) and apoB48 ($r = 0.117$; $p = 0.038$). HDL-C did not show any relationship with either FIB-4 or cIMT.

Conclusion: Subjects with RA at increased risk of liver fibrosis have more subclinical atherosclerosis. In RA, this relationship seems to be mediated by LDL-C, hypertension and accumulation of intestinal atherogenic remnants while the classical CVD risk factors in MASLD, triglycerides and HDL-C and systemic inflammation do not seem to play a role in the association between FIB-4 and RA.

PO7-02-YI

Lobular inflammation, but no NAS score, is associated with higher risk of progression to cirrhosis in patients with metabolic dysfunction-associated steatotic liver disease (MASLD)

Giordano Sigon[1 2], Roberta Forlano[1], Benjamin H. Mullish[1], Vicky Lin[1], Jian Huang[1], Chioma Izzi-Engbeaya[3], Aleksandra Dunin-Borkowska[3], Michael Yee[3], Robert D. Goldin[4], Mark Thursz[1], Pinelopi Manousou[1]
[1]Imperial College London, Liver unit/Division of Digestive Diseases, Department of Metabolism, Digestion and Reproduction, London, United Kingdom, *[2]University of Milan, Department of Pathophysiology and Transplantation, Milan, Italy*, *[3]Imperial College NHS Trust, Section of Endocrinology and Metabolic Medicine, St Mary's Hospital, London, United Kingdom*, *[4]Imperial College London, Department of Pathology, London, United Kingdom*
Email: gio.sigon@proton.me

Background and aims: Metabolic dysfunction associated steatotic liver disease (MASLD) is a growing issue in public health, reaching the record prevalence of 33% worldwide. MASLD has been related to both liver and non-liver related complications. However, little is known about how liver histology influences the clinical outcomes of these patients. Our aim is to evaluate the predictive value of histological features for clinical outcomes in patients with MASLD.

Method: Between 1/2013 and 12/2022, we consecutively included patients with biopsy-proven MASLD followed up in the liver clinic, St. Mary's Hospital, Imperial College Healthcare NHS Trust, London. At baseline, we collected information about demographic and anthropometric data, blood tests, comorbidities and histology. F3 was considered advanced fibrosis, whereas F4 cirrhosis (NASH CRN scoring system). Metabolic dysfunction associated steatohepatitis (MASH) was diagnosed as NAS ≥5. Clinical outcomes were recorded during follow-up and included major cardiovascular events (MACE: acute coronary syndrome, stroke or transient ischemic attach), new diagnosis of cirrhosis (either histologically or based on a combination of biochemical, radiological and elastography features), hepatic decompensation (first episode of variceal bleeding, ascites or encephalopathy), new extra-hepatic malignancies and death from all causes. Multivariate analysis was performed by COX regression using SPSS 26.

Results: 344 patients were included. Median age was 52 [42-61] years and median BMI 31 [27.6-35.0] kg/m2; 267 (64%) were men and 211 (51%) had T2DM. MASH was diagnosed in 111 patients (38%), F3 in 116 (34%), F4 in 68 (20%). Median follow-up was 5 [5-9] years. During follow-up, 19 (7%) patients developed cirrhosis. On multivariate analysis, F3 (HR: 3.9, 95%CI: 1.1-14.5, p = 0.04;) and moderate to severe lobular inflammation (HR 4.2, 95%CI: 1.6-11.4, p = 0.005) were associated with cirrhosis development. Moreover, 14 (4%) patients experienced hepatic decompensation. On multivariate analysis, only baseline F4 was associated (HR: 8.6, 95%IC: 1.9-38.6, p = 0.005) with decompensation. Furthermore, MACE occurred in 33 (3%) patients, whereas 17 (4%) patients developed extra-hepatic cancer. On multivariate analysis, only age was associated with risk of MACE and malignancies, whereas no association was found with any histological features. Finally, 29 (5%) patients died. On multivariate analysis, both age and baseline F4 were associated with higher risk of death from all causes.

Conclusion: Baseline liver fibrosis is associated with progression to cirrhosis and death from all causes in MASLD patients, whereas it is not related to risk of MACE and malignancies. Although the presence of steatohepatitis was not related to any clinal outcome, presence of moderate/severe lobular inflammation was associated with progression to cirrhosis.

PO7-04
Liver steatosis and fibrosis in severe obesity in diabetic and non-diabetic subjects: first report of the Franciscus obesity NASH Study

Willy Theel[1 2], Femme Dirksmeier-Harinck[3], Elisabeth van Rossum[2], Danny Kanhai[4], Jan Apers[5], Bas van Dalen[6], Bojou Neecke[7], Ellen van der Zwan-van Beek[8], Diederick Grobbee[9 10], Janneke Wiebolt[1], Manuel Castro Cabezas[1 2 10]
[1]Franciscus Gasthuis and Vlietland, Internal Medicine, Rotterdam, Netherlands, *[2]Erasmus MC, Internal Medicine, Rotterdam, Netherlands*, *[3]Franciscus Gasthuis and Vlietland, Gastroenterology and hepatology, Rotterdam, Netherlands*, *[4]Franciscus Gasthuis and Vlietland, Pediatrics, Rotterdam, Netherlands*, *[5]Franciscus Gasthuis and Vlietland, Bariatric surgery, Rotterdam, Netherlands*, *[6]Franciscus Gasthuis and Vlietland, Cardiology, Rotterdam, Netherlands*, *[7]Franciscus Gasthuis and Vlietland, Pathology, Rotterdam, Netherlands*, *[8]Franciscus Gasthuis and Vlietland, Clinical chemistry, Rotterdam, Netherlands*, *[9]Julius Center for Health Sciences and Primary Care, Utrecht, Netherlands*, *[10]Julius Clinical, Zeist, Netherlands*
Email: w.theel@franciscus.nl

Background and aims: The prevalence of Metabolic dysfunction Steatotic Fatty Liver Disease (MASLD) ranges from 25% in the general population to 90% in patients with obesity. MASLD can progress towards Metabolic dysfunction Steatohepatitis (MASH) which has been reported to be present in 26% of patients with severe obesity scheduled for bariatric surgery. The causes of MASLD and MASH are multifactorial but insulin resistance plays a central role. We investigated the prevalence and the severity of MASLD in patients enrolled for bariatric surgery in the Netherlands and the association with diabetes mellitus (DM).

Method: The Franciscus Obesity NASH Study (FONS) is a long-term observational prospective cohort study including patients scheduled for bariatric surgery. FONS will include 300 subjects. Here we present preliminary data for the first subset of patients. Transient elastography (TE) measurements using Fibroscan were performed before surgery to evaluate liver steatosis by Controlled Attenuation Parameters (CAP) and liver fibrosis with its elasticity (E) using Fibroscan. Liver biopsies were performed in patients of whom the Fibroscan measurements suggested significant fibrosis (≥8 KPa). Biopsies have been evaluated using the NAFLD Activity Score (NAS) and fibrosis staging.

Results: 137 Patients have been included in FONS at present. 81.2% were female and the mean (± SD) BMI was 42.95 (6.0) kg/m^2. DM was present in 15.3% (21 patients) and dyslipidemia in 33.6%. Mean CAP was 290.5 (50.4) dB/m and mean E was 7.87 (4.39) kPa. Only 10.9% had a CAP value considered to reflect severe steatosis (≥358 dB/m) and 37.3% an E value reflecting significant fibrosis (≥8 KPa), which is much lower than reported in the literature. In general, liver elasticity increased with BMI, waist circumference and liver steatosis as expected. Patients with DM were significantly older and they had a lower BMI of 39.07 (3.99) kg/m^2 vs. 43.65 (6.02) ($p < 0.001$) than those without DM. TE measurements were significantly higher in patients with DM (Table 1). Liver biopsies were performed following protocol in 20 subjects of whom 6 had DM. Five of these subjects (1 with DM) had MASH and the mean NAS was 1.15 (1.2). From the other 15 subjects only 2 biopsies showed steatosis and 3 biopsies showed inflammation. Nevertheless, significant fibrosis was found in 4 subjects, of whom 3 had DM.

Conclusion: These preliminary results of FONS confirm earlier results suggesting that patients with severe obesity and DM are potentially associated with increased risk of developing liver fibrosis . FONS will provide information on the use of FIB4 and Fibroscan to detect patients with severe obesity at risk of developing MASH and liver fibrosis. Long term follow-up data using Fibroscan will also provide information on the prognostic value of this device in a cohort with severe obesity.

Figure:

	Without DM	With DM	p value
n	116 (84.7%)	21 (15.3%)	
Age (years)	42 ± 11	47 ± 13	0.025
Female n (%)	97 (83%)	15 (71%)	0.183
Height (cm)	169 ± 8	169 ± 10	0.408
Weight (kg)	125.7 ± 20.8	112.7 ± 18.6	0.003
Waist Circumference (cm)	133 ± 14.2	126.9 ± 12.9	0.034
BMI	43.65 ± 6.02	39.07 ± 3.99	<0.001
CAP (dB/m)	284 ± 49	323 ± 42	<0.001
Elasticity (kPa)	7.47 ± 3.92	10.06 ± 6.05	0.006
IQR/med (%)	20.1 ± 6.8	18.5 ± 7.3	0.153

Table 1-Characteristics according diabetes status

Dara are presented as number (percentage) or mean ± SD.

CAP: controlled attenuation parameter, OSAS: obstructive sleep apnea, IQR/med: interquartile range median ratio.

PO7-05
Thermoacoustic assessment of fatty liver disease-a clinical feasibility study

Jang Hwan Cho[1], Michael Thornton[2], Jing Gao[3], Colby Adamson[3], Idan Steinberg[1]
[1]Endra Life Sciences, Ann Arbor, United States, *[2]Endra Life Sciences, London, Canada*, *[3]Rocky Vista University, Ultrasound Research medical center, Ivins, United States*
Email: thornto@gmail.com

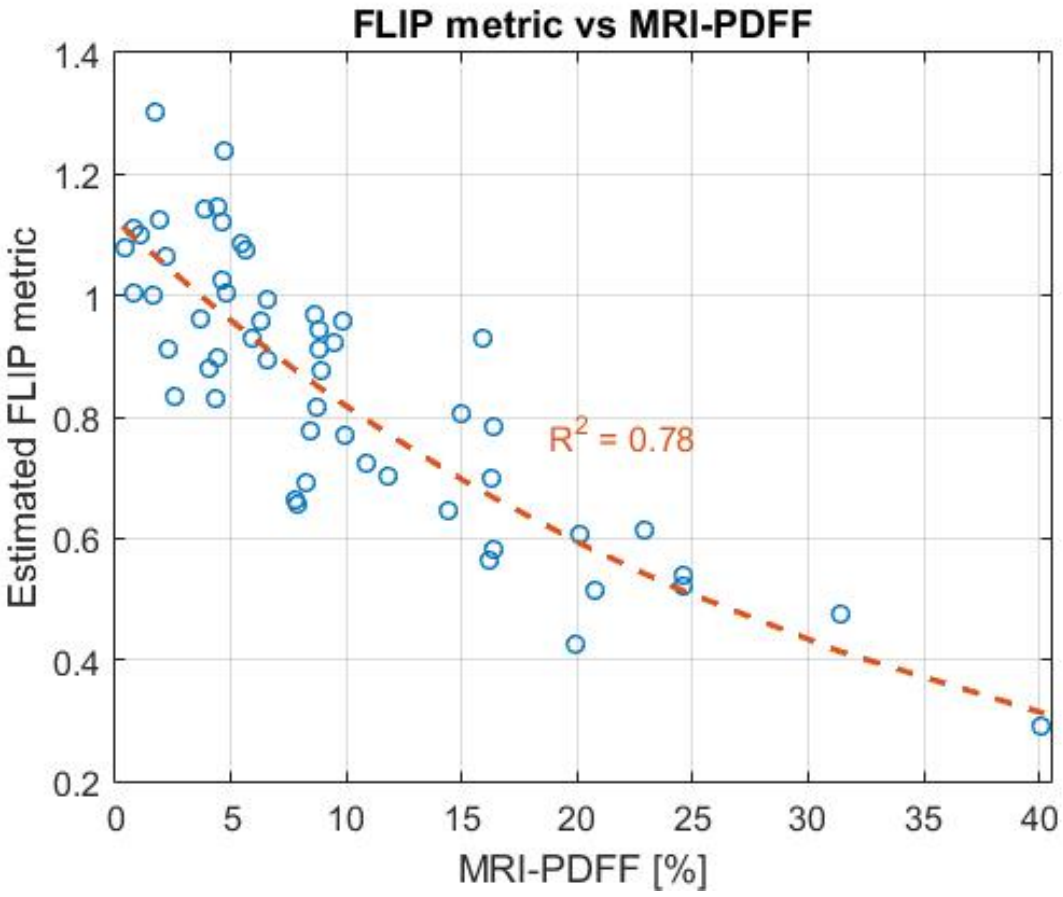
Background and aims: Ultrasound-based methods are the current standard of practice for point-of-care, non-invasive liver fat assessment. However, due to low penetration depth and confounding factors, invalid or poor-quality measurements are often encountered in large patients or patients with fibrosis. In contrast to purely ultrasound-based approaches, thermoacoustic (TA) approaches are sensitive to tissue composition chemistry rather than acoustic scattering and attenuation, making TA measurements of liver fat content relatively insensitive to liver fibrosis. Furthermore, the very low acoustic frequencies of TA signals (up to 1 MHz) allow for effective tissue penetration in large individuals. ThermoAcoustic Absorption Parameter (TAAP) derived from TA signals induced in tissue is proportional to the complex permittivity of the tissue and describes tissue conductivity. The Thermo-Acoustic Enhanced Ultrasound (TAEUS) Fatty Liver Imaging Probe (FLIP) is a hand-held, point-of-care device that quantitatively assesses liver fat and thus overcomes the drawbacks of conventional ultrasonic methods. This work describes clinical data obtained from two clinical sites and three operators, comparing TAEUS-FLIP with MRI-PDFF to assess fatty liver disease.

Method: 66 subjects with suspected Non-Alcoholic Fatty Liver Disease (NAFLD) participated in a study that included B-mode ultrasound, obtained by trained sonographers, to determine the locations of the liver capsule and overlying tissue (muscle, fat, and skin), followed by a TAEUS FLIP procedure consisting of 10 to 30 measurements. Finally, MRI-PDFF measurements were obtained to measure the subjects' true liver fat fraction. A TAEUS FLIP measurement consists of 80 pulses of 1-microsecond Radio Frequency (RF) pulses, each containing 5.7 milliJoules of energy. TA signals induced in the fat, muscle, and liver were normalized for energy distribution by using precalculated correction factors for each subject's known body habitus to estimate the conductivity of liver tissue. Using computerized simulation tools, these factors were derived from a wide range of body compositions (thin to obese). The median value was selected from a series of valid TAEUS FLIP measurements for each study participant to avoid outlier values. Linear regression was used to compare the TAEUS FLIP estimated liver conductivity with liver fat fraction measured by MR-PDFF. Sensitivity, specificity, and AUROC at 6% MRI-PDFF were determined by selecting the optimal TA measurement cut-off value.

Results: One subject had a failed TAEUS FLIP exam, and ten subjects with subcutaneous fat <6mm of subcutaneous fat were excluded. The 55 remaining subjects included 14 cases of fibrosis (confirmed by elastography) and a maximum BMI of 42. Figure 1 shows the median liver tissue conductivity for each subject compared to MRI-PDFF. The two methods are strongly correlated, with a correlation of $R^2 = 0.78$. The TAEUS FLIP method has a sensitivity of 0.88 and a specificity of 0.74 (AUROC = 0.93) in detecting the presence of fatty liver disease at a threshold of 6% MRI-PDFF.

Conclusion: This feasibility liver fat fraction study compares TAEUS-FLIP to MRI-PDFF and provides insight into the potential of TA methods to assess liver fat content, similar to MRI-PDFF, at the point of care.

Figure:


FLIP metric vs MRI-PDFF
Estimated FLIP metric
MRI-PDFF [%]
R^2 = 0.78
1.4
1.2
1
0.8
0.6
0.4
0.2
0
5
10
15
20
25
30
35
40

PO7-06
Risk factors for progression in metabolic dysfunction-associated steatotic liver disease

Letitia Toma[1 2], Simona Ioanitescu[1 2], Laurentiu Micu[1 2], Simu Razvan-Ioan[2], Mircea Istrate[2], Elena Laura Iliescu[1 2]
[1]Fundeni Clinical Institute, Internal Medicine, Bucharest, Romania, *[2]"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*
Email: letitia_toma@yahoo.com

Background and aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) is an important public health issue, associated with progression to cirrhosis and the need for liver transplantation. It is important to identify patients with increased risk of progression. This study aims to evaluate the importance of serum inflammatory markers in the progression of metabolic dysfunction-associated steatohepatitis (MASH), thus identifying patients at risk for significant fibrosis.

Method: We performed a prospective observational trial in 47 patients diagnosed with MASH, undergoing statin therapy for hyperlipemia. All patients had normal serum values of cholesterol and triglycerides on inclusion. Patients with other comorbidities were excluded from the study. We evaluated serum levels of adiponectin, leptin, interleukin-6, interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) on study inclusion. Liver biopsy was performed at the 2 evaluations.

Results: The mean age in the study group was 42.17± 13.59 years old, 59.5% were male. Initial biopsies revealed NAS score of 1A in 10 patients, 1B in 13 patients, 1C in 11 patients and 2 in 14 patients. At one year follow-up, we noted progression of NAS score in 8 patients: 2 patients from 1A to 1B, 3 patients from 1B to 1C, 1 patient from 1B to 2 and 4 patients from 2 to 3. Lower adiponectin levels, higher leptin, IL-6, IFN-γ and TNF-α were associated to progression of MASLD (Table 1).

Conclusion: Serum values of adiponectin, leptin, IL-6, IFN-γ and TNF-α can be used for identification of patients at risk for rapid progression of MASLD, in association with other risk factors for metabolic syndrome. These patients should take priority in clinical trials regarding MASLD progression and might be the most to benefit from strong therapeutic interventions.

Figure: Table 1 Serum parameters in patients with progression of MASLD

	Patients with progression of NAS score (N = 8)	Patients with similar NAS score (N = 39)	P =
Adiponectin (Median normal value Male: 7mg/L Female:8.5mg/L)	Male: 5.6 ± 1.9 Female: 6.4 ± 2.7	Male: 6.6 ± 1.2 Female: 9.1 ± 1.7	0.04 0.03
Leptin (% of upper normal limit for sex and body mass index)	139.56%	86.25%	0.01
IL-6 (N <7 pg/ml **)**	9.4 ± 2.6	5.2 ± 1.8	0.02
IFN-γ (N <0.1 UI/ml[6])	0.26 ± 0.08	0.08 ± 0.03	0.03
TNF-α (N <8.1pg/ml[2])	14.27 ± 3.19	5.93 ± 2.46	0.01

PO7-08
Novel compound SNP-6 series active metabolites mitigate non-alcoholic steatohepatitis and fibrosis: results from preclinical models and a phase 2a clinical trial

Chang-Hui Tsao[1], Hsin-Tien Ho[2], Yu-Lueng Shih[3], Tien-Yu Huang[3], Wen-Hui Fang[4], Chang-Hsien Liu[5], Jung-Chun Lin[3], Te-Yu Lin[6], Chih-Weim Hsiang[5], Kai-Min Chu[2], Cheng-Huei Hsiong[2], Yung-En Wu[2], Jia-Yu Hao[2], Guan-Ju Chen[2], Yi-Hsuan Lin[2], Jun-Lan Zeng[2], Fu-Yi Hsieh[2], Chih-Wen Liang[2], Yoa-Pu Hu[7 8]
[1]Sinew Pharma Inc, Taipei, Taiwan, *[1]Sinew Pharma Inc, Taipei, Taiwan*, *[3]Tri-Service General Hospital, National Defense Medical Center, Division of Gastroenterology, Taipei, Taiwan*, *[4]Tri-Service General Hospital, National Defense Medical Center, Division of Family Medicine, Taipei, Taiwan*, *[5]Tri-Service General Hospital, National Defense Medical Center, Division of Radiology, Taipei, Taiwan*, *[6]Tri-Service General Hospital, National Defense Medical Center, Division of Infectious, Taipei, Taiwan*, *[7]National Defense Medical Center, School of Pharmacy, Taipei, Taiwan*, *[8]Taipei Medical University, Taipei, Taiwan*
Email: changhui.tsao@sinewpharma.com

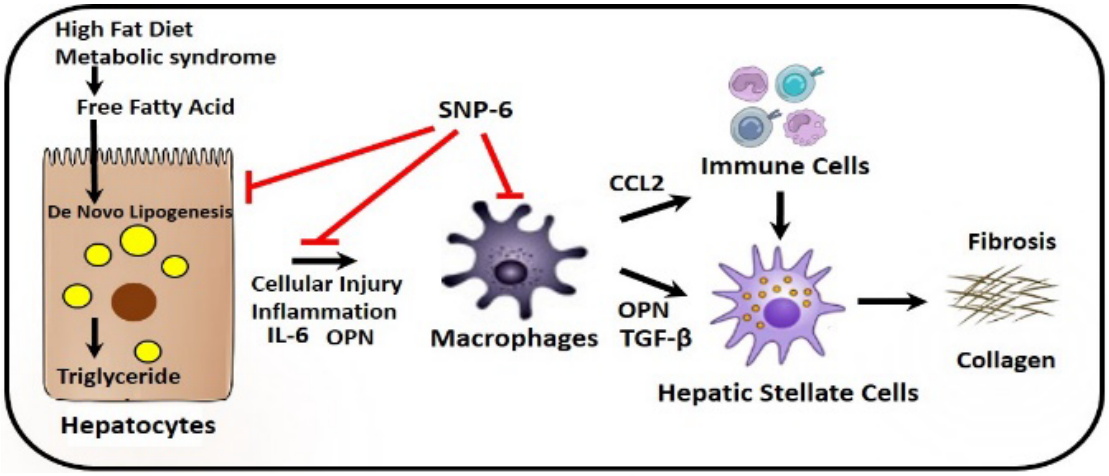
Background and aims: Non-alcoholic steatohepatitis (NASH) is a growing global health concern, yet there is a lack of effective pharmacological treatment for this disease. The objective of this study is to investigate the potential of SNP-630, a newly developed synthetic molecule with multiple mechanisms of action, and its active metabolites SNP-612 to alleviate liver injury resulting from NASH. The study intends to evaluate the efficacy of SNP-630 and SNP-612 in hepatocytes and macrophages, mouse model and NASH patients, with the aim of identifying a drug candidate that can target multiple pathways implicated in NASH.

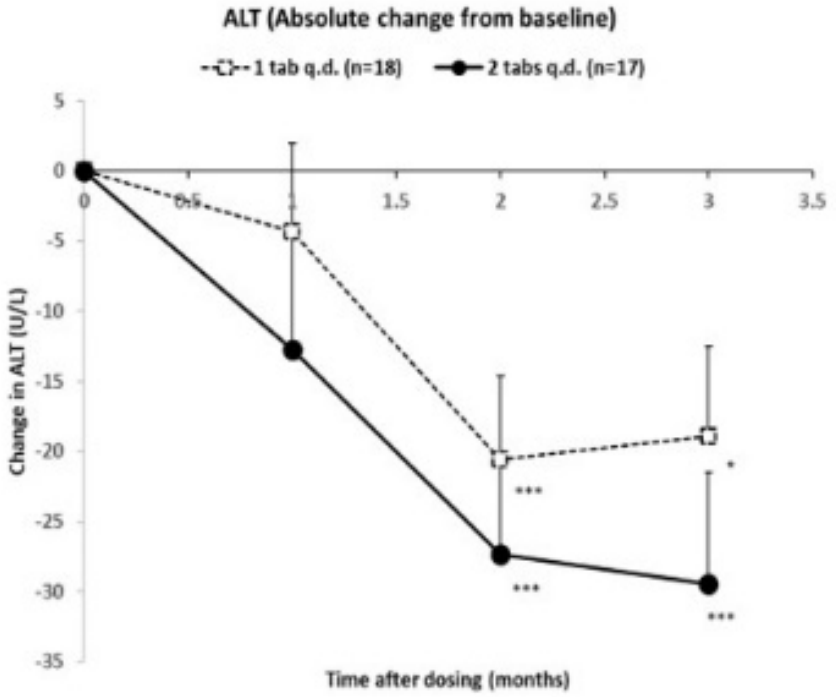
Method: HepG2 were treated with palmitic acid or with SNP-630/or SNP-612 for 24h, and then, HepG2-derived conditioned medium (CM) were harvested and subjected to treat human macrophages THP-1 cell line, which is differentiated with phorbol-12-myristate-13-acetate (PMA), for another 24h. RNA were then harvested and subjected to qPCR analysis. [*In vivo* murine NASH model] Male C57BL/6 mice were fed with a high-fat diet (HFD) for 21 weeks and then, SNP-630 was administered orally while continuing the HFD for an additional 10 weeks. In prevention model, male C57BL/6 mice were fed with a HFD combined with SNP-612 for 21 weeks. [Phase 2a clinical trial] The tolerability, safety, and efficacy of SNP-612 were evaluated in 35 NASH patients. The primary and secondary end points were the changes in serum aminotransferase (ALT) level and liver steatosis, inflammation and fibrosis, respectively, from baseline to week 12.

Results: In CM experimental model, the expression of pro-inflammatory cytokine IL-6 and CCL2, and pro-fibrotic protein osteopontin were significantly decreased in THP-1 treated with SNP-630-treated lipid-loaded HepG2-derived CM. In line with *in vitro* model, SNP-630 and SNP-612 significantly alleviate NASH by improving hepatic steatosis (liver triglycerides and cholesterol), reducing liver damage and inflammation (the level of ALT and CCL2), and mitigating hepatic fibrosis (the expression of *Col3a1* and *Timp1*). Consist with our preclinical data, patients treated with SNP-612 exhibited a significant and dose-dependent reduction in serum ALT levels at week 12 compared to baseline. In addition, significant reductions were also noticed in the circulating levels of chemokines (CCL4 and CCL5), which are involved in liver inflammation and fibrosis. Moreover, liver stiffness, as measured by Fibroscan, also showed a significant decrease in patients with F4 fibrosis stage who received SNP-612 treatment. Finally, no serious adverse event has been noted in this trial.

Conclusion: The finding of this study highlight the novel compound SNP-630 and its active metabolites SNP-612 in mitigating hepatic steatosis, inflammation and fibrosis through diverse mechanisms (i.e. targeting of hepatocyte and macrophage) in cellular and murine model of NASH. Additionally, we provide evidence for the efficacy and safety of SNP-612 in NASH patients. These results demonstrate the therapeutic potential of SNP-630 as promising candidates for the treatment of NASH.

Figure: Mechansims of Action of SNP-6 (B) ALT value changes from baseline





PO7-09
Clinical and biochemical features of diagnosed and undiagnosed patients with non-alcoholic fatty liver disease

Julia Uhanova[1], Gerald Minuk[1]
[1]*University of Manitoba, Internal Medicine, Winnipeg, Canada*
Email: julia.uhanova@umanitoba.ca

Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a common condition that affects 20-30% of the world's population, however the majority of those affected remain undiagnosed. Clinical and biochemical features of NAFLD are largely derived from studies involving patients who had been diagnosed and/or referred to tertiary care centres for further management. Whether these features apply to individuals with undiagnosed NAFLD has not been determined. The aim of this study was to document and compare the demographics, associated metabolic co-morbidities, liver biochemistry and non-invasive markers of hepatic fibrosis and portal hypertension in diagnosed versus undiagnosed NAFLD patients.

Method: The two study cohorts consisted of 3, 101 NAFLD patients attending a tertiary care centre (diagnosed) and 408 individuals with NAFLD identified as a result of their volunteering in a community-based NAFLD screening clinic (undiagnosed).

Results: In the entire study population before matching, the diagnosed NAFLD patients were younger (51 ± 14 versus 55 ± 13 years, $p < 0.00001$), more often male (50% versus 34%, $p < 0.00001$) and diabetic (40% versus 17%, $p < 0.00001$) but less often dyslipidemic (29% versus 46%, $p < 0.00001$). BMIs were similar in the two cohorts. In the matched analysis, the prevalence and extent of liver transaminases (ALT and AST) and function test (albumin, bilirubin and INR values) abnormalities were greater in diagnosed NAFLD patients as were non-invasive determinants of hepatic fibrosis and portal hypertension (FIB-4 and low platelet counts respectively). To balance the uneven numbers of the two cohorts, the analysis was repeated on a subset of undiagnosed and age/sex matched 1:2 diagnosed cases with largely the same results.

Conclusion: The results of this study indicate that diagnosed and undiagnosed NAFLD patients are two different patient populations. The demographics, metabolic, and disease severity profiles differ significantly in diagnosed versus undiagnosed NAFLD patients.

Table: Metabolic and biochemical measures and abnormalities in diagnosed and undiagnosed NAFLD patients

	Diagnosed (N=3101)			Undiagnosed (N=408)			
Viariable	**N**	**Mean±SD**	**Median**	**N**	**Mean±SD**	**Median**	**P**
ALT IU/L	2,700	70±68	54	341	28±17	23	<0.00001
AST IU/L	2,700	49±54	36	340	23.1±9.9	20	<0.00001
ALP IU/L	2,700	101±64	87	336	77±23	76	<0.00001
GGT IU/L	2,700	120±185	62	338	36±41	23	<0.00001
T.Bili µmol/L	2,700	11±30	8	340	7.9±3.8	7	0.05
Albumin g/L	2,700	40.1±4.9	40	340	44.4±3.9	45	<0.00001
INR	2,700	1.05±0.61	1	241	1.00±0.19	1	0.005
Platelets	2,111	239±78	236	233	256±62	246	0.0008
FIB-4	2,676	1.5±2.8	1	231	1.1±0.9	0.71	0.036
Prevalence	**N**	**n**	**%**	**N**	**N**	**%**	**P**
Diabetes, %	3,098	1,240	40	408	70	17	<0.00001
Dyslipidemia, %	3,098	908	29	408	187	46	<0.00001
Obesity, %	879	620	71	386	272	70	0.98
ALT <40 IU/L	2,700	905	34	341	280	82	<0.00001
ALT >1.5N	2,700	1,588	59	341	39	11	<0.00001
AST >1.5N	2,700	811	30	340	13	3.8	<0.00001
ALP >1.5N	2,700	149	5.5	341	0	0	0.00003
GGT >1.5N	2,700	1,446	54	338	46	14	<0.00001
T.Bill >N	2,700	71	2.6	340	0	0	0.0045
Alb <N	2,700	119	4.4	339	2	0.6	0.001
INR >N	2,700	50	1.9	241	2	0.8	0.369
Low Platelets	2,111	217	10%	226	6	2.70%	0.0003
FIB-4 >1.35	2,676	876	33%	225	46	20%	0.0001

PO7-13-YI
Validation of the enhanced liver fibrosis (ELF)-test in heparinized and EDTA plasma for future reflex testing in diagnostic algorithms

Koen van Son[1], Anne-Marieke van Dijk[2], Anne Linde Mak[2], Julia Witjes[2], Veera Houttu[2], Diona Zwirs[2], Max Nieuwdorp[2], Bert-Jan van den Born[2], Johan Fischer[3], Henrike Hamer[4], Ulrich Beuers[5], Joanne Verheij[6], Onno Holleboom[2]
[1]Radboudumc, Gastroenterology and Hepatology, Nijmegen, Netherlands, *[2]Amsterdam UMC, Vascular medicine, Amsterdam, Netherlands*, *[3]Amsterdam UMC, Public and occupational health, Amsterdam, Netherlands*, *[4]Amsterdam UMC, Laboratory medicine, Amstedam, Netherlands*, *[5]Amsterdam UMC, Gastroenterology and hepatology, Amsterdam, Netherlands*, *[6]Amsterdam UMC, Pathology, Amsterdam, Netherlands*
Email: k.c.vanson@amsterdamumc.nl

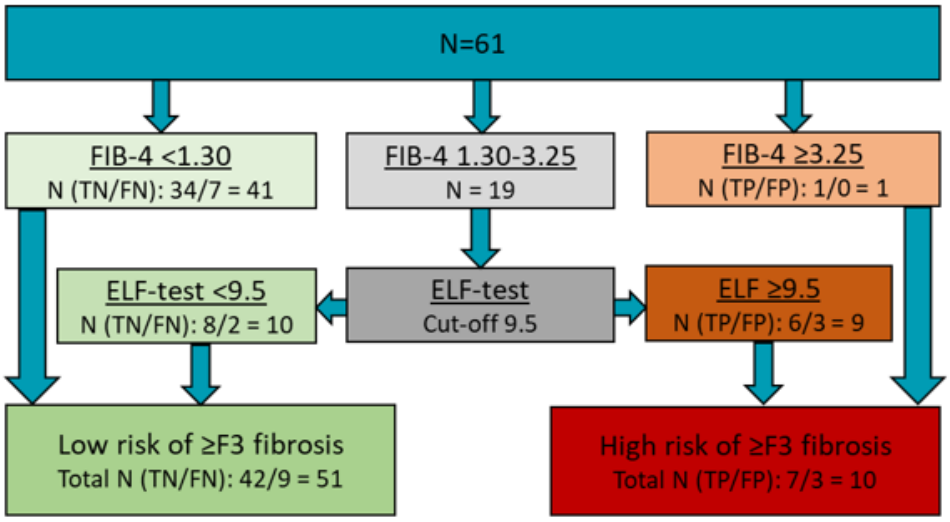
Background and aims: The rising prevalence and severity of metabolic dysfunction associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), requires diagnostic algorithms to non-invasively detect advanced liver fibrosis. Implementation of such algorithms may be greatly enhanced with reflex testing: for example, when the widely implementable Fibrosis-4 (FIB-4) test is within the intermediate range and therefore inconclusive, an Enhanced Liver Fibrosis (ELF)-test is automatically conducted. Hampering such implementation, AST, ALT included in FIB-4 are regularly measured in heparinized or EDTA plasma while the ELF-test is standardized for serum. Here we studied the use of ELF-test in both heparinized and EDTA plasma to facilitate its use in reflex testing.

Method: 143 participants were included: 80 individuals at risk for MASLD from the general population and 63 individuals with histologically-proven MASLD from an academic clinic. Controlled attenuation parameters (CAP) and Liver Stiffness Measurements (LSM) using vibration controlled transient elastography (VCTE) were performed in all participants as a proxy for steatosis and fibrosis, respectively. The results of ELF-test in heparinized and EDTA plasma were compared to those in serum. The sensitivity and specificity of a FIB-4/ELF two-tiered reflex test algorithm, in which ELF-test follows an intermediate FIB-4 score (1.30-3.25), was determined in the histologically characterized cohort. A cut-off of 9.5 was used for ELF-test.

Results: Median age was 55.0 [44.0; 66.5] years and mean BMI was 33.5 (SD 5.4) kg/m^2. Median CAP and LSM were 321 [263; 366] dB/m and 7.2 [4.9; 9.5] kPa, respectively. 21.3% of the histologically characterized population had F3 fibrosis and 6.6% had cirrhosis. Mean ELF-score in heparinized plasma (9.24 (0.84)) did not differ significantly from serum (9.09 (0.84), p = 0.146), while ELF-score in EDTA (mean 8.76 (0.84)) was significantly lower than in serum (p = 0.001). The mean of each of the three individual ELF proteins (hyaluronic acid (HA), procollagen III amino terminal peptide (PIIINP) and tissue inhibitor of metalloproteinase 1 (TIMP-1)) in heparinized and EDTA plasma differed from serum. Therefore, corrective formulas were designed to calculate a corrected ELF-score in heparinized and EDTA plasma. The sensitivity and specificity of the FIB-4/ELF reflex test in heparinized plasma for advanced MASLD fibrosis (≥F3) were 43.8% and 93.3%, respectively.

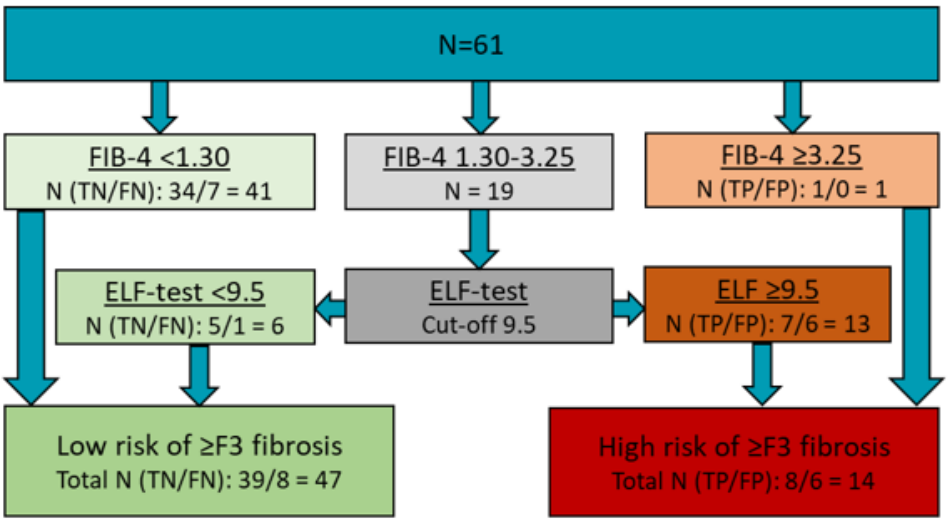
Conclusion: ELF-score in heparinized plasma was comparable to serum, yet in EDTA, ELF-score was lower. The value of the three ELF proteins determined in heparinized and EDTA plasma differed from those determined in serum. After validation in an independent cohort, the correction formulas allow for implementation of reflex testing with FIB-4 and ELF-test for MASLD fibrosis.

Figure:



FN = false negative, FP = false positive, TN = true negative, TP = true positive

1a



FN = false negative, FP = false positive, TN = true negative, TP = true positive

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Figure 1: Flowchart of reflex testing with FIB-4 and ELF-test measured in serum (a) and measured in heparinized plasma (b) with liver biopsy as reference standard

PO7-16-YI
Glucagon-like peptide-1 receptor agonists and risk of major adverse liver outcomes in patients with chronic liver disease and type 2 diabetes

Axel Wester[1], Ying Shang[1], Emilie Toresson Grip[1], Anthony Matthews[2], Hannes Hagström[1]
[1]Karolinska Institutet, Department of Medicine Huddinge, Stockholm, *[2]Karolinska Institutet, Unit of Epidemiology, Institute of Environmental Medicine*
Email: axel.wester@ki.se

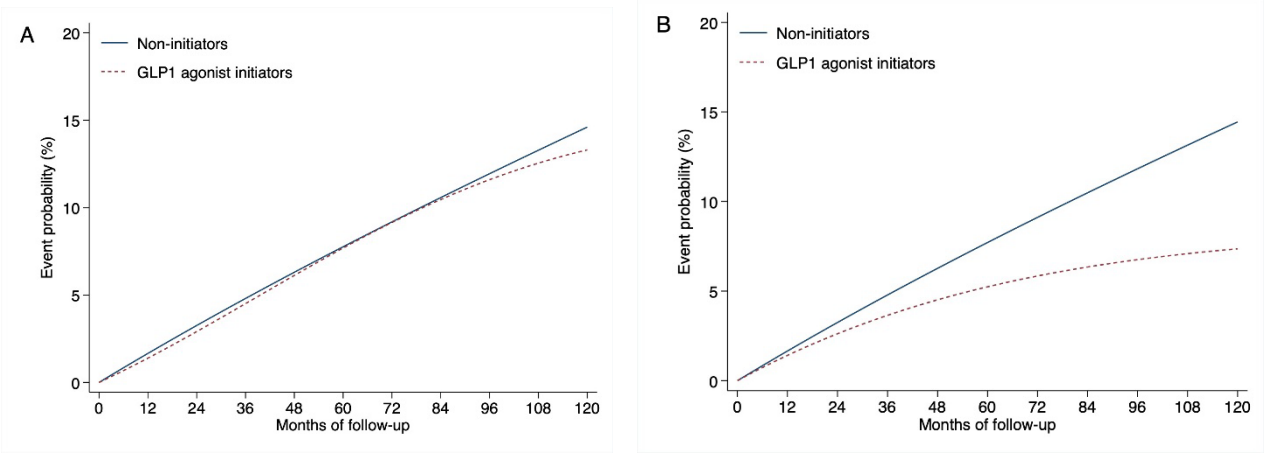
Background and aims: Phase II trials suggest glucagon-like peptide-1 receptor (GLP1) agonists resolve non-alcoholic steatohepatitis, but do not affect regression of fibrosis. We aimed to determine the long-term causal effect of GLP1 agonists on the risk of major adverse liver outcomes (MALO) in patients with any chronic liver disease and type 2 diabetes.

Method: We used observational data from Swedish healthcare registers between 2010 and 2020 to emulate a target trial of GLP1 agonists in eligible patients with chronic liver disease and type 2 diabetes. We used an inverse probability weighted marginal structural model to compare estimates of 10-year MALO risk (decompensated cirrhosis, hepatocellular carcinoma, liver transplantation, or MALO-related death) in initiators of GLP1 agonists with non-initiators.

Results: Initiators of GLP1 agonists (n = 1, 026) had a 10-year risk of MALO at 13.3% compared to 14.6% in non-initiators (n = 15, 633) in the intention-to-treat analysis (risk ratio [RR] = 0.91, 95% confidence interval [CI] = 0.50-1.32). The corresponding risk estimates in the per-protocol analysis were 7.4% and 14.4%, respectively (RR = 0.51, 95%CI = 0.14-0.88). There was no effect of GLP1 agonists in patients with non-alcoholic fatty liver disease (438 initiators; 4, 618 non-initiators; intention-to-treat RR = 1.41, 95%CI = 0.53-2.30) or compensated cirrhosis (161 initiators; 1, 869 non-initiators; intention-to-treat RR = 1.05, 95%CI = 0.20-1.91).

Conclusion: In patients with chronic liver disease and type 2 diabetes who adhered to therapy over time, treatment with GLP1 agonists resulted in a lower risk of MALO. This suggests that GLP1 agonists are promising agents to reduce risk of chronic liver disease progression in patients with concurrent type 2 diabetes, although this needs to be corroborated in randomized clinical trials.

Figure: Inverse probability weighted risk curves of major adverse liver outcomes comparing initiators of glucagon-like peptide-1 receptor (GLP1) agonists with non-initiators. A: intention-to-treat effect, B: per-protocol effect.



PO7-17
The efficacy of multi-disciplinary lifestyle modifications in Taiwanese non-alcoholic steatohepatitis patients- subgroup analysis of the placebo group patients in a randomized trial

Jee-Fu Huang[1], Ming-Lun Yeh[1], Chung-Feng Huang[1], Chia-Yen Dai[1], Ming-Lung Yu[1 2], Wan-Long Chuang[1]
[1]Kaohsiung Medical University, Department of Internal medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, *[2]National Sun Yat-Sen University, School of Medicine, College of Medicine, Kaohsiung, Taiwan*
Email: jf71218@gmail.com

Background and aims: Fibrosis progression is a major predictive factor of patient outcome in non-alcoholic steatohepatitis (NASH) patients. We aimed to investigate the paired histopathological features of the Taiwanese NASH patients randomized into placebo group in a double-blind randomized trial.

Method: All the biopsy-proven NASH patients received a 6-month strict multi-disciplinary program of lifestyle modifications led by physician, dietician, and nursing staff. The histopathological and clinical features obtained from paired biopsies 24 weeks apart were assessed. The end points were normalization of transaminase levels, metabolic parameters, decrease of NAFLD Activity Score (NAS) ≥1, and fibrosis stage decrease ≥1 stage. We also aimed to elucidate the predictors associated with disease progression.

Results: A total of 37 biopsy-proven NASH patients were consecutively enrolled into placebo arm of the trial. The normalization of transaminase level increased from 0% to 13.5%. There were also significantly increased proportions of patients with normal total cholesterol, triglyceride, and hemoglobin A1c levels. Fifteen (40.5%) patients had increased NAS ≥1, whereas 10 (27.0%) patients had NAS regression, respectively. Twelve (32.4%) patients had increased fibrosis ≥1 stage. Only 2 (5.4%) patients had fibrosis regression. A high fasting plasma glucose (FPG) level was associated with NAS progression. Older age, higher transaminase and FPG levels were factors associated with fibrosis progression. rGT and FPG levels were associated with NAS progression. Those patients with NAS progression had increased BMI, hip/waist circumference, AST and ALT levels after the program as compared to those without NAS progression. There were 7 (18.9%) patients achieving body weight reduction >3%, and 4 (57.1%) of them had NAS regression.

Conclusion: Taiwanese NASH patients had a significant disease progression in a period of six months. A more precise or intensive program may be needed for disease control.

PO7-18-YI
Predictive ability of non-invasive fibrosis tests for long-term liver, cardiovascular and kidney outcomes in Europeans with metabolic risk factors from the UK Biobank

Federica Tavaglione[1 2], Antonio De Vincentis[2 3], Oveis Jamialahmadi[4], Raffaele Antonelli Incalzi[2 3], Antonio Picardi[1 2], Stefano Romeo[4 5 6], Umberto Vespesiani Gentilucci[1 2]
[1]Fondazione Policlinico Universitario Campus Bio-Medico, Clinical Medicine and Hepatology Unit, Rome, Italy, *[2]Università Campus Bio-Medico di Roma, Department of Medicine and Surgery, Rome, Italy*, *[3]Fondazione Policlinico Universitario Campus Bio-Medico, Internal Medicine Unit, Rome, Italy*, *[4]University of Gothenburg, Department of Molecular and Clinical Medicine, Sahlgrenska Academy, Gothenburg, Sweden*, *[5]Sahlgrenska University Hospital, Cardiology Department, Gothenburg, Sweden*, *[6]University Magna Graecia, Clinical Nutrition Unit, Department of Medical and Surgical Sciences, Catanzaro, Italy*
Email: fede.tavaglione@gmail.com

Background and aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) is the leading cause of chronic liver disease worldwide. MASLD is associated with both liver-related and extrahepatic complications, including cardiovascular disease (CVD) and chronic kidney disease (CKD). Non-invasive tests (NITs) for advanced fibrosis are increasingly used to identify individuals with MASLD who are at risk for liver-related complications. Their predictive ability for extrahepatic complications has been recently tested in tertiary care settings and found to be limited. Herein, we investigated the performance of NITs for predicting long-term liver, cardiovascular and kidney outcomes in individuals with dysmetabolism from the large prospective UK Biobank**.**

Method: First, we selected Europeans with overweight/obesity and/or type 2 diabetes. Next, we selected at baseline 1) 305, 745 individuals without any liver disease, 2) 194, 236 individuals without chronic viral hepatitis and CVD, and 3) 203, 522 individuals without chronic viral hepatitis and CKD. Then, we estimated the performance of NITs for predicting 1) incident progressive liver disease (PLD: cirrhosis, decompensated liver disease, hepatocellular carcinoma, liver transplantation), 2) incident CVD (angina, myocardial infarction, stroke, transient ischemic attack), and 3) incident CKD (eGFR<60 ml/min/1.73m^2, chronic renal failure, kidney transplant status) by Cox proportional hazards models. Follow-up length was calculated from the date of baseline assessment visit up to the first date of target outcome diagnosis, the date of death, or the date of end of follow-up at the assessment center (July 1st, 2022), whichever occurred first. The following NITs were tested: fibrosis-4 index (FIB-4), NAFLD fibrosis score (NFS), fibrotic NASH index (FNI), AST to platelet ratio index (APRI) and BARD.

Results: After a median follow-up of 9 years, FNI was the best score for predicting liver outcomes (area under the curve [AUC] 0.77, p <0.05 vs all the other NITs). After a median follow-up of 13 years, NFS was the best score for predicting cardiovascular outcomes (AUC 0.60, p <0.05 vs all the other NITs). After a median follow-up of 13 years, NFS was the best score for predicting kidney outcomes (AUC 0.73, p <0.05 vs all the other NITs). All NITs showed a worse and limited performance for extrahepatic outcomes compared to liver outcomes, except for a slightly better performance of NFS and BARD for kidney outcomes.

Conclusion: NITs showed a satisfactory predictive ability for liver outcomes, but a rather modest predictive ability for cardiovascular and kidney outcomes in individuals with dysmetabolism from the general population.

Figure:

	PLD (N = 305, 745)		CVD (N = 194, 236)		CKD (N = 203, 522)	
Score	**AUC**	**P value**	**AUC**	**P value**	**AUC**	**P value**
FIB-4	0.75 (0.73-0.77)	0.03	0.59 (0.59-0.60)	<0.001	0.62 (0.60-0.64)	<0.001
NFS	0.72 (0.70-0.74)	<0.001	0.60 (0.60-0.61)	reference	0.73 (0.71-0.75)	reference
FNI	0.77 (0.75-0.79)	reference	0.59 (0.58-0.60)	0.003	0.64 (0.63-0.66)	<0.001
APRI	0.75 (0.73-0.77)	0.01	0.53 (0.53-0.54)	<0.001	0.52 (0.50-0.54)	<0.001
BARD	0.62 (0.61-0.64)	<0.001	0.55 (0.54-0.55)	<0.001	0.65 (0.63-0.67)	<0.001

PO7-19
Role of cuprotosis-related genes along the spectrum of metabolic dysfunction-associated fatty liver disease

Haiyang Yuan[1], Wen-Yue Liu[2], Gong Feng[3], Sui-Dan Chen[4], Xin-Zhe Jin[5], Zi-Jun Song[6], Chris Byrne[7], Giovanni Targher[8], Gang Li[9], Xin-Lei Zhang[1], Na Tian[1], Jacob George[10], Ming-Hua Zheng[1 11 12]
[1]the First Affiliated Hospital of Wenzhou Medical University, Department of Hepatology, *[2]the First Affiliated Hospital of Wenzhou Medical University, Department of Endocrinology*, *[3]Xi'an Medical University*, *[4]the First Affiliated Hospital of Wenzhou Medical University, Department of Pathology*, *[5]the First Affiliated Hospital of Wenzhou Medical University, Department of Laboratory Medicine*, *[6]Zhejiang University School of Medicine, The First Affiliated Hospital*, *[7]Southampton General Hospital, Southampton National Institute for Health and Care Research Biomedical Research Centre*, *[8]University of Verona, Section of Endocrinology, Diabetes and Metabolism, Department of Medicine*, *[9]Jining No.1 People's Hospital, Department of Infectious*, *[10]Westmead Hospital and University of Sydney, Storr Liver Centre*, *[11]Wenzhou Medical University, Institute of Hepatology*, *[12]Key Laboratory of Diagnosis and Treatment for The Development of Chronic Liver Disease in Zhejiang Province*
Email: zhengmh@wmu.edu.cn

Background and aims: Metabolic dysfunction-associated fatty liver disease (MAFLD) is the most common form of chronic liver disease and an important cause of hepatocellular carcinoma (HCC). Cuprotosis is a newly discovered form of copper-dependent programmed cell death. However, its role in the development and progression of MAFLD has not been elucidated. We explored the associations between cuprotosis-related genes (CRGs) across different stages of liver disease in MAFLD, including HCC.

Method: We analysed four datasets of MAFLD (GSE135251, GSE48452, GSE185051, GSE162694) and three of MAFLD-related HCC (GSE192959, GSE193066, GSE103080). To investigate the associations between CRGs and cuprotosis, we performed differential analyses, correlation analyses, logistic regression, and functional enrichment analyses. We validated the findings in an independent cohort (PERSONS) which was also used for a genome-wide association study (GWAS).

Results: GLS, SOD1, SOD2 and BAX expression accurately identified significant liver fibrosis in patients with MAFLD (Figure A). Amongst the genes, SOD1 was negatively associated with fibrosis while the other three were positively associated with fibrosis. GLS expression showed significant differences across the whole MAFLD spectrum (false discovery rate, FDR<0.05), and identified significant fibrosis in patients with MAFLD-related HCC (Figure B). Functional enrichment analysis further revealed the role of GLS in the progression of MAFLD to MAFLD-related HCC (Figure C). GWAS and expression quantitative trait loci (eQTL) analysis revealed that multiple SNPs of both GLS and SOD2 genes were associated with clinical indices of MAFLD. For example, Several SNPs of GLS, such as rs11675981, rs12998878, were significantly correlated with the presence or absence of both significant fibrosis and advanced fibrosis (Table 1).

Conclusion: Cuprotosis may play an important role in the progression of MAFLD and provides new insights on MAFLD pathogenesis.

Figure:

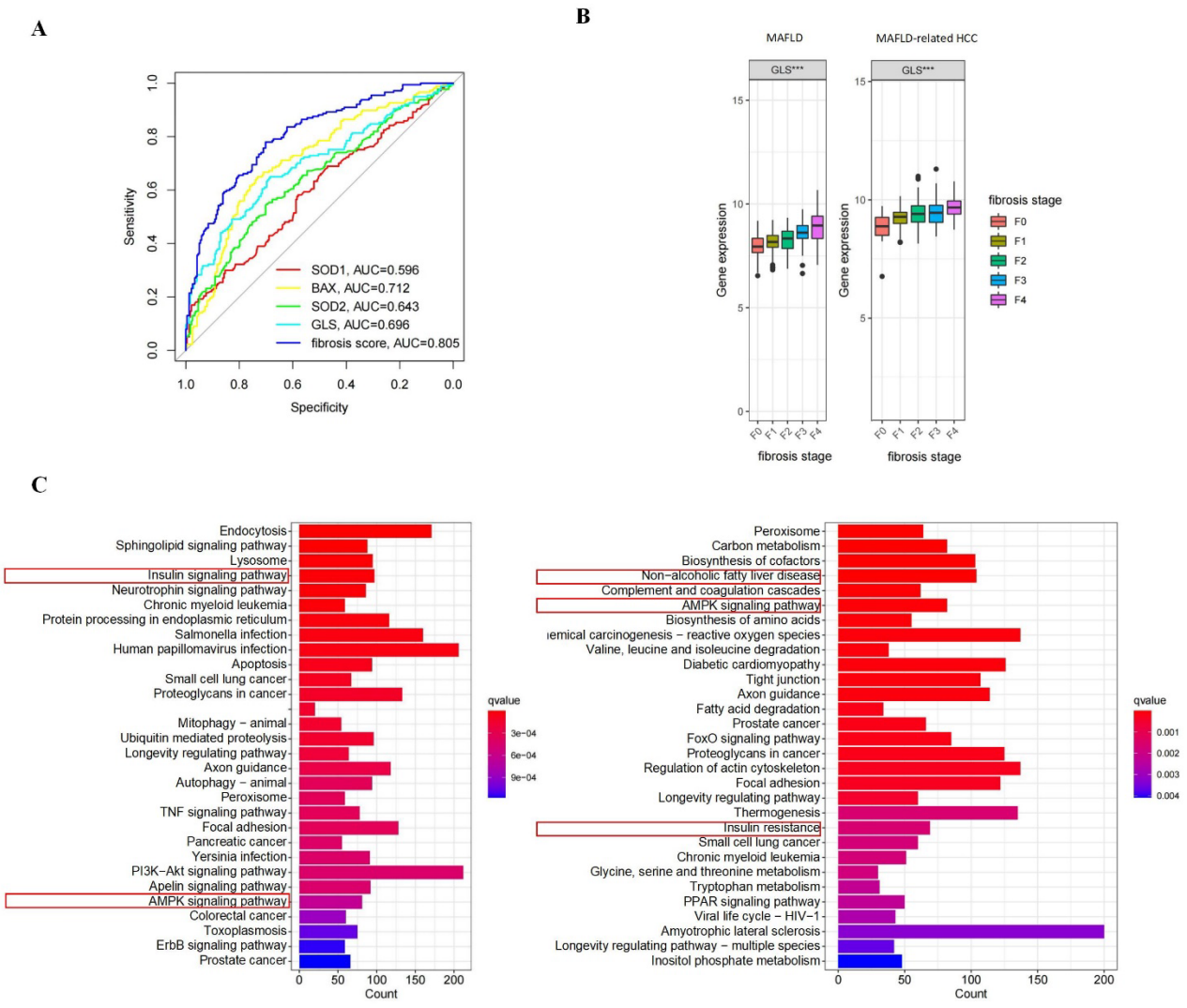

A
Sensitivity
Specificity
SOD1, AUC=0.596
BAX, AUC=0.712
SOD2, AUC=0.643
GLS, AUC=0.696
fibrosis score, AUC=0.805
B
MAFLD
MAFLD-related HCC
GLS***
Gene expression
fibrosis stage
F0
F1
F2
F3
F4
C
Endocytosis
Sphingolipid signaling pathway
Lysosome
Insulin signaling pathway
Neurotrophin signaling pathway
Chronic myeloid leukemia
Protein processing in endoplasmic reticulum
Salmonella infection
Human papillomavirus infection
Apoptosis
Small cell lung cancer
Proteoglycans in cancer
Mitophagy − animal
Ubiquitin mediated proteolysis
Longevity regulating pathway
Axon guidance
Autophagy − animal
Peroxisome
TNF signaling pathway
Focal adhesion
Pancreatic cancer
Yersinia infection
PI3K−Akt signaling pathway
Apelin signaling pathway
AMPK signaling pathway
Colorectal cancer
Toxoplasmosis
ErbB signaling pathway
Prostate cancer
Count
qvalue
Peroxisome
Carbon metabolism
Biosynthesis of cofactors
Non−alcoholic fatty liver disease
Complement and coagulation cascades
AMPK signaling pathway
Biosynthesis of amino acids
Valine, leucine and isoleucine degradation
Diabetic cardiomyopathy
Tight junction
Axon guidance
Fatty acid degradation
Prostate cancer
FoxO signaling pathway
Proteoglycans in cancer
Regulation of actin cytoskeleton
Focal adhesion
Longevity regulating pathway
Thermogenesis
Insulin resistance
Small cell lung cancer
Chronic myeloid leukemia
Glycine, serine and threonine metabolism
Tryptophan metabolism
PPAR signaling pathway
Viral life cycle − HIV−1
Amyotrophic lateral sclerosis
Longevity regulating pathway − multiple species
Inositol phosphate metabolism
Count
qvalue

Table: Results of associations between SNPs of GLS and MAFLD-related indicators.

SNP(s)	Gene	Feature	A1[a]	A2[b]	MAF[c]	Trait	β	P-value
rs11675981	GLS	intronic	C	T	0.19	advF	0.592221262	0.03519
						sigF	0.359072069	0.02903
rs11688967	GLS	intronic	G	A	0.1322	advF	0.746213901	0.01426
rs12987113	GLS	intronic	A	G	0.133	advF	0.740507752	0.01509
rs12990256	GLS	intronic	A	G	0.1474	advF	0.722220428	0.01623
rs12992618	GLS	intronic	A	G	0.1324	advF	0.744790414	0.01448
rs12998878	GLS	intronic	A	G	0.19	advF	0.592221262	0.03519
						sigF	0.359072069	0.02903
rs13024969	GLS	intronic	A	C	0.1322	advF	0.746213901	0.01426
rs1921907	GLS	intronic	G	A	0.133	advF	0.740507752	0.01509
rs2108818	GLS	intronic	C	G	0.1474	advF	0.722220428	0.01623
rs3771313	GLS	intronic	C	T	0.2044	advF	0.578297339	0.03670
rs66799711	GLS	intronic	A	AAC	0.1474	advF	0.722220428	0.01623
rs6713444	GLS	intronic	A	G	0.189	advF	0.598836501	0.03303
						sigF	0.36741704	0.02572
rs6743496	GLS	intronic	G	A	0.1474	advF	0.722220428	0.01623
rs6758866	GLS	intronic	G	A	0.1474	advF	0.722220428	0.01623
rs71030313	GLS	intronic	AT	A	0.133	advF	0.740507752	0.01509
rs984610	GLS	intronic	C	T	0.1467	advF	0.728031582	0.01538

[a]A1: reference allele [b]A2: other allele [c]MAF: Minor allele frequency of A2
Abbreviations: SNP, single nucleotide polymorphism; MAF, mutation annotation format; advF, advanced fibrosis; sigF, significant fibrosis.

POSTER ABSTRACT PRESENTATIONS

NURSES & ALLIED HEALTH PROFESSIONALS

PO3-07
Behavioural specifics of patients living with non-alcoholic fatty liver disease/metabolic dysfunction-associated steatotic liver disease-a key to improve communication

Andreas Geier[1], Jörn Schattenberg[2], Andreas Teufel[3], Rainer Guenther[4], Alisan Kahraman[5], Ali Canbay[6], Wolf Peter Hofmann[7], Heide Möller-Slawinski[8], Paula Nießing[9], Nicola Nowak[10], Achim Kautz[9], Diane Langenbacher[9]
[1]University Hospital Würzburg, Department of Internal Medicine II, Würzburg, Germany, *[2]Universitätsmedizin Mainz, I. Medizinische Klinik, Mainz, Germany*, *[3]Universitätsklinikum Mannheim, II. Medizinische Klinik, Mannheim, Germany*, *[4]Universitätsklinikum Schleswig-Holstein, Klinik für Innere Medizin I, Kiel, Germany*, *[5]Max Grundig Klinik GmbH, Bühl, Germany*, *[6]Universitätsklinikum Knappschaftskrankenhaus Bochum GmbH, Medizinische Klinik, Bochum, Germany*, *[7]Gastroenterologie am Bayerischen Platz, Berlin, Germany*, *[8]SINUS Markt- und Sozialforschung GmbH, Heidelberg, Germany*, *[9]Kautz5 gUG, Köln, Germany*, *[10]Dr. Nowak Consulting, Frankfurt am Main, Germany*
Email: geier_a2@ukw.de

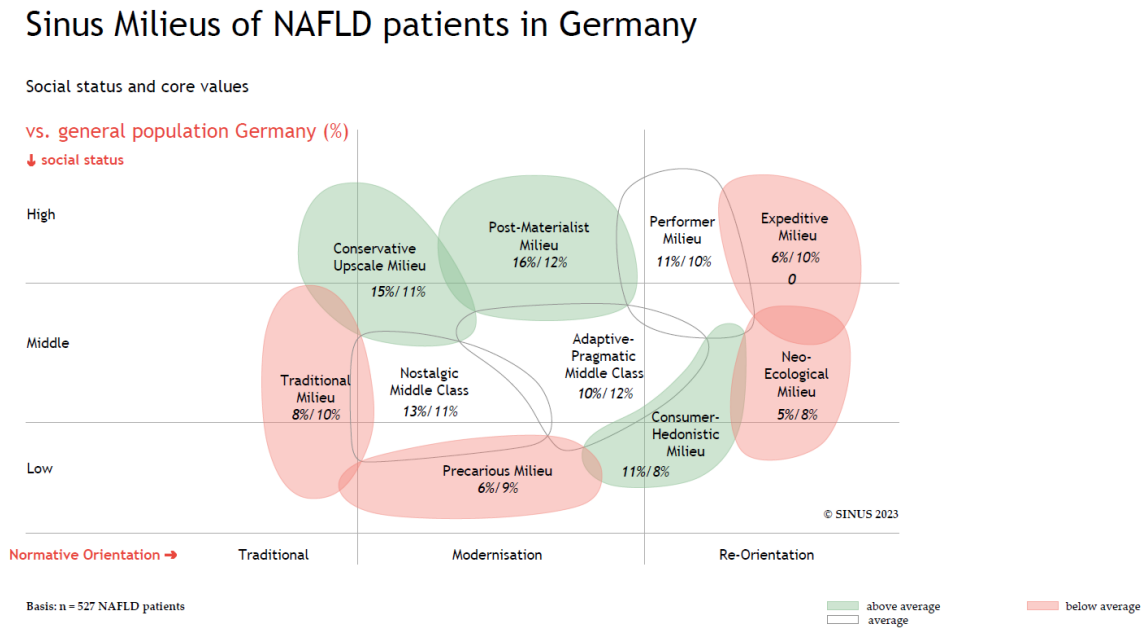
Background and aims: Non-alcoholic fatty liver disease (NAFLD) (now termed MASLD), is an increasing public health issue in Europe with estimations of over 25% (3-5% having metabolic-associated fatty liver disease (MASH)[i]. Our research is analysing the detailed behavioural specifics of MASLD patients by clustering them to groups of like-minded people, determined by similar lifestyle and preferences, similar value orientation and social situation, similar communication and behaviour, so called Sinus-Milieus.

Method: 2420 anonymised questionnaires have been provided to 24 hospital and office based hepatologists to get distributed to diagnosed NAFLD patients. The validated Sinus Institute methodology[ii] has been used to perform the cohort´s milieu allocation, extended by a set of additional questions about the disease, co-morbidities and lifestyle patterns.

Results: 527 responses from 11 centres have been collected within a study duration of 18 months (22% response rate). Basic characteristics of the cohort were 51% females, mean age 55.9 years mean BMI 3257; self-reported co-morbidities 40% diabetes type II, 58% hypertension, 54% hypercholesterolemia and 8% cardiovascular events. Mean FIB 4 was 2.07 with 4% >3, 25 overrepresented in the upper and middle social class milieus. The cohort matches well with data from the German NAFLD registry[iii] and the FLAG study[iv] thereby validating these self-reported data. Of note, 6% of responders indicated alcohol consumption above 20g (women) and 30g (men) with an overrepresentation in the lower social class milieus.In comparison to the milieu allocation in the general population NAFLD/MASLD are overrepresented in the Consumer-Hedonistic, Post-Materialist and Conservative-Upscale Milieu (see figure). Milieu allocations in subgroups such as individuals doing more than 3 hours of sports per week or sticking to healthy nutrition provide valuable insights for effective counselling and communication. The general knowledge of liver parameters is significantly lower compared to other liver diseases[v] with the low social class milieus being at the lowest end. Empowered patients may be more present in the upper and middle social class milieus.

Conclusion: This is the first ever research looking into milieu related specifics of NAFLD/MASLD patients. Unexpectedly, there are all milieus represented, especially those with high affinity to health topics in the upper and middle social class. The knowledge about milieu provides a basis for more effective communication strategies (e.g., quick milieu allocation checklists and communication tools) between doctors and NAFLD/MASLD patients. The current findings provide strong evidence to bring the principles of differentiated patient communication into the scientific dialog in NAFLD/MASLD.

Figure:


Sinus Milieus of NAFLD patients in Germany
Social status and core values
vs. general population Germany (%)
social status
High
Middle
Low
Conservative Upscale Milieu
15%/ 11%
Post-Materialist Milieu
16%/ 12%
Performer Milieu
11%/ 10%
Expeditive Milieu
6%/ 10%
0
Traditional Milieu
8%/ 10%
Nostalgic Middle Class
13%/ 11%
Adaptive-Pragmatic Middle Class
10%/ 12%
Neo-Ecological Milieu
5%/ 8%
Consumer-Hedonistic Milieu
11%/ 8%
Precarious Milieu
6%/ 9%
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Normative Orientation
Traditional
Modernisation
Re-Orientation
Basis: n = 527 NAFLD patients
above average
average
below average

PO4-19-YI
Depression, anxiety, worry and emotional dysregulation in compensated advanced chronic liver disease

Eleonora Maggio[1], Antonio Liguori[1], Luigi Andrea Antuofermo[1], Giuseppe Marrone[1], Marco Biolato[1], Maurizio Pompili[1], Antonio Gasbarrini[1], Rosaria Calia[1], Luca Miele[1]
[1]Fondazione Policlinico Universitario A. Gemelli IRCCS-Università Cattolica del Sacro Cuore, Rome, Italy
Email: luca.miele@policlinicogemelli.it

Background and aims: Persistent depressive symptoms predict worse health-related quality of life in Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD). In addition, anxiety presents clear associations with progression of chronic liver diseases. No previous study evaluated the role of emotional regulation and worry in MASLD patients. We aim to investigate the association between some psychopathological factors and Compensated Advanced Chronic Liver disease (cACLD) in MASLD patients.

Method: We conduct a cross-sectional study at A. Gemelli's Hospital Steatotic Liver Disease (SLD) Clinic from January 2023 in the PROMETEO cohort (NCT04371042). The study group consists of 98 patients with MASLD divided into two groups (cACLD/no-cACLD) according to Baveno VII guidelines. Each patient underwent a psychological interview with the administration of the following self-report validated questionnaires: BDI-II (depression), STAI-Y (state and trait anxiety), PSWQ (pathological worry), DERS (difficulties in the ability to identify/regulate emotions). Descriptive analyses on the sample and Student's *t* tests for all the scales/subscales of psychological testing were performed according to cACLD diagnosis.

Results: Among the 98 patients (mean age: 54.02 years; 67.3% men; mean BMI: 30.3 Kg/m^2) 27/98 (27.6%) were cACLD, 35/98 (35.7%) reach the clinical cut-off for depression, 15/98 (15.3%) for state/trait anxiety, 15/98 (15.3%) for emotional dysregulation, and 58/98 (59.2%) for uncontrollable and excessive worry. The effect of scales of general depression, somatic-affective component of depression, state anxiety and trait anxiety is statistically significant ($p < .05$), where the cACLD group has higher depressive and anxious symptomatology than no-cACLD group. cACLD group has higher DERS-Awareness ($p < .01$) and lower DERS-Strategies ($p < .05$) than non-cACLD group. Moreover, cACLD group reported significantly more psychiatric disorders during lifetime ($p < .05$) and refer more motivation towards psychological support for their disease ($p < .01$).

Conclusion: Patients with cACLD show more depression, anxiety, and emotional dysregulation than patients without cACLD. They also have more emotional awareness but, on the other hand, limited access to emotion regulation strategies. Therefore, they have insight on their disease but lack strategies to cope with it, so it is possible that they somatize their difficulties with anxiety and depression. Therefore, it is important to plan a psychological intervention with the aim of improving therapeutic adherence and, in turn, to hamper the clinical evolution of the disease.

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