EASL
The Home of Hepatology

FIRST
NAFLD SUMMIT

ABSTRACTS

TARGET-ORIENTED APPROACH TO DIAGNOSIS AND PHARMACOTHERAPY OF NASH, A DIALOGUE BETWEEN ACADEMIA AND INDUSTRY

09-11 NOVEMBER 2017
ROME, ITALY

INVITED SPEAKERS' ABSTRACTS

02

Benefits and perils of promoting lipid combustion or inhibiting lipogenesis (DGAT, SCD, ANGPTL-3 inhibitors) to counteract hepatic lipotoxicity

Leanne Hodson* [1]

[1]*Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, United Kingdom*

Corresponding author's email: leanne.hodson@ocdem.ox.ac.uk

It remains unclear why intra-hepatocellular fat starts to accumulate but it is likely to involve an imbalance between fatty acid delivery to the liver, fatty acid synthesis and oxidation within the liver, and triglyceride export from the liver. *De novo* lipogenesis (DNL) is the process whereby excess non-lipid precursors are synthesised to fat and in humans this primarily occurs in the liver. Despite being energetically inefficient and likely a minor route for storing excess energy, it is often suggested that enhanced DNL is related to the pathogenesis of non-alcoholic fatty liver disease (NAFLD). The primary end product of DNL is the saturated fatty acid palmitoyl-CoA, which may interfere with cellular function. Within hepatocytes there are two diacylglycerol acyltransferases (DGAT1 and DGAT2) which are abundantly expressed in the same cells and belong to unrelated protein families. Both catalyse the final step in triglyceride synthesis, thus triglyceride can initially be formed in two ways: 1) via the DGAT1 pathway through esterification of exogenous fatty acids, and 2) via the DGAT2 pathway which utilises DAG derived from *de novo* synthesised fatty acids as substrates. It has been suggested that triglyceride predominantly synthesised by DGAT1 is considered metabolically 'good', whilst that synthesised by DGAT2 is considered metabolically 'bad'; thus inhibition of DGAT2 could be metabolically advantageous. In addition, DNL and stearoyl CoA desaturase (SCD1), the rate-limiting enzyme in the synthesis of monounsaturated fatty acids, appear to be intimately linked. There is evidence to suggest DNL-derived fatty acids are preferentially channelled into pathways of elongation and desaturation in hepatocytes. It has been hypothesised that hepatic SCD1 activity may be involved in the pathophysiology of NAFLD in humans. Importantly, the entry of fatty acyl-CoA into the mitochondrion for oxidation is dependent on carnitine palmitoyltransferase 1 (CPT1); malonyl-CoA, an intermediate in the DNL pathway, is a potent inhibitor of this. Individuals with NAFLD are reported to have lower hepatic fatty acid oxidation, thus up-regulation of this pathway, potentially via inhibition of the DNL pathway, may promote greater disposal of intra-hepatocellular fatty acids and ultimately lower the risk of developing NAFLD. The potential benefits and perils of inhibiting lipogenesis and/or promoting oxidation to counteract hepatic lipotoxicity will be discussed.

Disclosure of Interest: None Declared

04

PPARalpha, delta and FGF21 as pharmacological targets for NASH

Bart Staels[*1]

[1]*University of Lille & Pasteur Institute of Lille, Lille, France*

Corresponding author's email: bart.staels@pasteur-lille.fr

Non-alcoholic fatty liver disease (NAFLD) is a liver pathology with increasing prevalence due to the obesity epidemic. Hence, NAFLD represents a rising threat to public health due to hepatic and cardiovascular complication. Currently, no effective treatments are available to treat NAFLD and its complications such as cirrhosis and liver cancer. Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear receptors which regulate lipid and glucose metabolism as well as inflammation. In this presentation, we will review recent findings on the pathophysiological role of the PPARalpha and delta isoforms in the different stages of NAFLD, from steatosis to NASH and fibrosis, as well as the preclinical and clinical evidences for potential therapeutical use of PPAR agonists in the treatment of NASH. We will also discuss the role of their downstream target FGF21 in NASH, potentially through its effects on brown adipose tissue and white adipose tissue beiging. Clinical trials are currently assessing the clinical benefits of these approaches in humans.

Disclosure of Interest: B. Staels: Consultant / Advisor: Genfit SA

05

CAP, MR and MR spectroscopy– does hepatic lipid content / composition matter

Manuel Romero-Gomez* [1]

[1]*Digestive Diseases, Virgen del Rocio University Hospital, Seville, Spain*

Corresponding author's email: mromerogomez@us.es

Liver fat infiltration is very common on liver diseases across different aetiologies from viral hepatitis to autoimmune hepatitis or hemochromatosis and especially in patients with NAFLD where fat is a diagnostic criterion. The infiltration of fat in the liver could be detected by ultrasonography, using transient elastography (controlled attenuated parameter (CAP)) or using magnetic resonance, mainly spectroscopy and proton density fat fraction.

a) **Ultrasonography** is easy to use, comfortable and detects liver steatosis as hyperechogenicity. However, the main limitation is the inaccuracy when fat infiltration is under 30% of hepatocytes that unfortunately is a common situation.

b) **Controlled attenuated parameter** (CAP) is based on the propagation of ultrasound waves across the liver. These waves propagate at a frequency of around 3.5 mega-Hertz (MHz) when CAP is used in combination with a standard transient elastography device. The acoustic impedance (the resistance of the propagated wave) reflects the viscoelastic characteristics of the liver. The degree of ultrasound attenuation correlates with hepatic fat infiltration, related to fat droplets in the cytoplasm of the hepatocytes that influence the viscoelasticity of the liver. However, CAP can measure changes in the liver beyond fat infiltration as the metabolic syndrome expression in the liver. Karlas et al (Karlas et al. J Hepatol 2017;66:1022-1030) combined data from 19 studies conducting a strong collaborative project, which included 2735 cases with histology and CAP analysis. This amount of data allowed the authors to conduct a robust statistical analysis demonstrating the usefulness of CAP in the quantification of hepatic steatosis. They defined cut-offs, variables influencing the output such as BMI, diabetes and aetiology and solved discrepancies between CAP and liver fat infiltration by histology.

c) **Magnetic resonance**
MR system could utilize signal fat fraction as an easy process giving an indirect marker of fat infiltration. Two signal fat fraction techniques have been reported: Fat suppressed technique and Chemical Shift technique. Proton density fat fraction is more complex, but a direct measure of fat infiltration with higher diagnostic accuracy.

MR spectroscopy measures several compounds in the liver including water and lipids. The peaks could be quantified as the under curve area and compared. In the liver, water peak is identified at 4.26 ppm but lipid peaks are identified at 0.9, 1.3, 2.0, 2.2, and 5.3 ppm. These peaks represent CH_3 (0.9 ppm), (CH_2) (1.3 ppm), CH_2 (2.0 and 2.2 ppm), and CH (5.3 ppm) lipids. The dominant lipid peaks are caused by the resonance of methyl (-CH_3) protons and methylene (-CH_2) in the triglyceride molecule, at 0.9–1.1 ppm and 1.3–1.6 ppm, respectively, along the frequency domain. Imaging biomarkers to detect liver steatosis matches with some key features recommended but not all of them; a) acceptability by patients, b) excludes bias in the process of measure, c) cost, d) acceptable diagnostic accuracy, e) errors are well controlled, f) a reliable method, g) availability. Body fat composition beyond liver infiltration is a key aspect in the improvement of the knowledge of NAFLD. Several methods have been suggested but many limitations emerged to block their implementation in clinical practice: 1.- Air displacement Plethysmography (ADP) could measure whole body adipose tissue; 2.- Dual-energy X-ray absorptiometry (DXA) could measure whole body adipose tissue but it is

an ionizing radiation; 3.- Computed Tomography (CT) is an ionizing radiation too; 4.- MR spectroscopy is useful for quantification of liver fat infiltration but requires local expertise for data analysis; 5.- Proton density Fat fraction is an option able to quantify fat in the whole body, visceral adipose tissue, abdominal subcutaneous adipose tissue and liver fat without safety concerns about radiation nor requirement of local expert for data analysis.
MR spectroscopy is the most accurate method to quantify liver fat infiltration but is time consuming, requiring post-processed action and could be complex in some cases. Currently, PDFF is the most common method to quantify fat in the liver.

The gold standard utilized in these studies was the quantification of steatosis by liver biopsy, the feature of NASH that reached the highest agreement between pathologists as has been demonstrated (Ratziu et al. Gastro 2005;128:1898-1906). Steatosis is graded in 4 from steatosis 0 (S0) when <5% of hepatocytes with fat droplets; S1 between 5% and 33%; S2 between 33% and 66% and S3 when higher than 67%. The key point in steatosis relevance was linked to the association with fibrosis progression increasing risk of liver cirrhosis and liver cancer. “Normal” steatosis seems to be when lower than 5% of hepatocytes with fat droplets or MR spectroscopy lower than 5.56%.

Lipid content is crucial to know the pathogenic role of these lipids on the disease. Thousands of lipid species have been related to progression of the disease from NAFLD to NASH and fibrosis. Phospholipids, triacylglycerols & non-esterified fatty acids were quantified significantly different in patients with NAFLD versus patients with NAFLD and fibrosis. Moreover, the hepatic lipidome of NAFLD is characterized by low phosphatidylcholine, high lysophosphatidylcholine and high free cholesterol (Alonso et al. Gastro 2017;157:1449). Thus, to quantify liver fat infiltration and to characterize the composition of lipid in the fat droplets are mandatory for the correct management of NAFLD.

Disclosure of Interest: None Declared

07

Academic overview: What dissociates steatosis and insulin resistance in humans?

Hannele Yki-Jaervinen[*1]

[1]*Department of Medicine, University of Helsinki, Helsinki, Finland*

Corresponding author's email: Hannele.Yki-Jarvinen@helsinki.fi

Although steatosis is often a marker of insulin resistance, liver triglycerides (TGs) themselves are inert and do not cause insulin resistance. NAFLD associated with features of insulin resistance ('Metabolic NAFLD') is characterized by hyperglycemia, hyperinsulinemia, hyperTG, hypoadiponectinemia and adipose tissue insulin resistance, while NAFLDs caused by the I148M variant in PNPLA3 ('PNPLA3 NAFLD') or the E167K variant in TM6SF2 ('TM6SF2 NAFLD') are not. These 3 types of NAFLD provide excellent human models to study mechanisms dissociating steatosis and insulin resistance. We profiled the human liver lipidome in 125 subjects undergoing liver biopsy. The subjects were divided into equally sized groups with 'Metabolic NAFLD' and 'No Metabolic NAFLD' based on their median HOMA-IR, and into groups of 'PNPLA3 NAFLD' (carriers of the I148M gene variant) and 'No PNPLA3' (no I148M gene variant). Liver TGs were similarly increased in 'Metabolic NAFLD' (15 vs. 5%) and 'PNPLA3 NAFLD' vs. respective control groups. Also, NASH was equally frequent in 'Metabolic NAFLD' (29 vs 11%) and 'PNPLA3 NAFLD' (28 vs 12%). Liver TGs in 'Metabolic NAFLD' were enriched with saturated and monounsaturated TGs while in 'PNPLA3 NAFLD' liver TGs were polyunsaturated. Ceramides, which cause insulin resistance and are formed from saturated fatty acids, were markedly increased in 'Metabolic NAFLD' but not in 'PNPLA3 NAFLD'. The increase in ceramides was due to that in de novo ceramide synthesis since dihydroceramides but no other ceramide synthetic precursors were increased. Ceramides and dihydroceramides containing a saturated (16:0, 18:0) fatty acyl chain were particularly increased, consistent with two recent independent studies in mice identifying C16:0 ceramide as the principal mediator of obesity-related insulin resistance. These data show that the composition of human liver TGs depends on the etiology. In 'Metabolic NAFLD', the liver is enriched with saturated TGs and ceramides containing saturated fatty acyl side chains. These data together with 5 recent prospective studies identifying ceramides as independent predictors of cardiovascular disease suggest that ceramides might predispose individuals with 'Metabolic NAFLD' not only to insulin resistance but also its cardiovascular sequelae.

Disclosure of Interest: None Declared

08

Insulin resistance: insulin sensitisers and related agents as treatments for NAFLD

Christopher Byrne* [1]

[1]*University of Southampton, Southampton, United Kingdom*

Corresponding author's email: c.d.byrne@southampton.ac.uk

There is a strong link between excessive deposition of triglyceride in liver that defines non-alcoholic fatty liver disease (NAFLD) and decreased sensitivity to the biological actions of insulin (insulin resistance). Insulin sensitivity is defined as the reciprocal of insulin resistance and when insulin resistance occurs in patients with NAFLD it is often manifest because it occurs with simple features of the metabolic syndrome (MetS). These simple features of the MetS employ thresholds of waist circumference, blood pressure, fasting glucose and triglyceride concentrations and high density lipoprotein cholesterol, (e.g. dyslipidaemia, dysglycaemia, increased blood pressure and central obesity) and when three or more of the features are present that defines the presence of the syndrome.

Although patients with NAFLD frequently have MetS, defining and identifying whether they have insulin resistance is much more challenging. In patients with NAFLD, insulin resistance frequently occurs not only in the liver, but also in other key insulin sensitive tissues such as skeletal muscle and adipose tissue. The gold standard for assessing whole body insulin sensitivity is the hyperinsulinaemic euglycemic clamp, and for assessing hepatic insulin sensitivity is the low dose insulin clamp that also utilises deuterated glucose in order to assess hepatic glucose production. Assessing insulin sensitivity in adipose tissue is similarly challenging and rather than study insulin-mediated glucose uptake into adipose tissue, investigators tend to assess the effects of endogenous insulin production to suppress serum non esterified fatty acid concentrations during the oral glucose tolerance test. Consequently, assessing insulin sensitivity is difficult, time consuming and expensive; and therefore to circumvent these problems, investigators resort to assessing HOMA-IR for assessing insulin resistance and QUICKI (the reciprocal of HOMA-IR) for assessing insulin sensitivity. Both of these measurements involve a ratio of fasting insulin concentration and fasting glucose concentration, and therefore require that pancreatic beta cell function is not impaired, otherwise this will lead to a misleading insulin to glucose ratio. Beta cell function is often impaired when patients with type 2 diabetes have had the condition for several years. Since a high proportion of patients with NAFLD have type 2 diabetes, an estimation of insulin resistance that requires measurement of fasting insulin concentrations, may be inaccurate in patients with NAFLD who have type 2 diabetes.

When liver lipid accumulates, components of the lipid biosynthesis pathway such as di-acyl glycerol and ceramides accumulate and these lipid moieties impair efficient insulin signalling (causing insulin resistance). Excessive/ectopic fat depositions in the liver could be due to increased fatty acid delivery from adipose tissue, increased synthesis of fatty acid via the de novo pathway, increased dietary fat, decreased mitochondrial beta oxidation, decreased clearance of very low density lipoprotein (VLDL) particles, or these factors in combination. It is still a matter of debate whether insulin resistance causes NAFLD or, whether excessive accumulation of lipids, or precursors on the synthetic pathway precede a diagnosis of NAFLD, and promote insulin resistance.

There is evidence that increased delivery of non-esterified fatty acid (NEFA) to the liver from the peripheral (adipose) tissue is important in the development of NAFLD. Some investigators, have reported that approximately 60% of fat deposited in the hepatocytes is generated from adipose tissue sources, and evidence is accumulating that hepatic lipid accumulation is also capable of promoting

hepatic inflammation and thereby the development of non-alcoholic steatohepatitis. Thus, when considering potential therapies for NAFLD, and what may be responsible for insulin resistance or inflammation in NAFLD, it is not only important to consider insulin actions and modifiers of insulin secretion such as glucagon like peptides (GLPs), but also the linked consequences of these effects in insulin sensitive tissues beyond the liver, such as adipose tissue and skeletal muscle.

There has been considerable interest recently in 'insulin sensitizing' therapies and modifiers of insulin (e.g. GLP-1), as novel treatments for NAFLD. However, when considering the effects of these therapies, there are many different pathways, not only in the liver, but also in other tissues, whose actions indirectly affect the liver. This presentation will discuss, insulin resistance, what causes it and how it can be measured, and the different types of 'insulin sensitizing' drugs, and modifiers of insulin, that have been tested, and their effects in NAFLD. The presentation will also attempt to rationalize the relative successes and failures of these agents by understanding how they act in NAFLD.

Disclosure of Interest: None Declared

09

GLP-1 receptor agonists in the diabetes and obesity pipeline: A potential in NASH?

Lotte B. Knudsen[*1]

[1]*Global Research, -Novo Nordisk, -Maaloev, Denmark*

Corresponding author's email: lbkn@novonordisk.com

Glucagon-Like Peptide-1 (GLP-1) was originally described as an incretin hormone. GLP-1 receptor agonists have been developed into a widely used treatment option for type 2 diabetes, with a mechanism of action that includes increased insulin secretion and reduced glucagon secretion, both in a glucose-dependent manner providing an effective injectable treatment with little risk of hypoglycaemia, and additional weight loss. The GLP-1 receptor has a physiological role in both glucose homeostasis and appetite regulation. The GLP-1 receptor agonist class of agents today include six different products which can be divided into a short-acting and a long-acting sub-class. Efficacy and mechanism of action is different between the two, where short-acting agents have less overall efficacy on HbA1c but have a larger specific effect on the incremental post-prandial glucose load. For the long-acting agents (defined as having active pharmacological plasma levels for 24 hours/day) there are significant differences in weight loss efficacy, where the fatty acid acylated long-acting GLP-1R agonist liraglutide that is also approved separately for the obesity indication has been shown to lead to more weight loss than all the other long-acting GLP-1 receptor agonists, in large randomised controlled clinical trials. The weight loss mechanism is mediated via brain GLP-1 receptors and is primarily an effect to reduce energy intake and may also involve altered reward signalling, leading to a change in food choice. Semaglutide is an investigational drug that has completed phase 3 clinical testing for the type 2 diabetes indication. Semaglutide is designed based on the same basic technology as liraglutide but is substantially optimized to have stronger and more specific albumin binding, higher potency and better brain uptake, the latter thought to be the mechanism behind the superior clinical efficacy now reported in several large randomized controlled clinical trials. Besides type 2 diabetes, semaglutide is also in clinical development for the obesity and the NASH indication. A randomised controlled clinical study has shown a beneficial effect of liraglutide in NASH. Both liraglutide and semaglutide have been shown to reduce cardiovascular risk in type 2 diabetes patients in two randomised controlled cardiovascular trials involving 9340 and 3297 patients, respectively. The mechanism behind the reduced cardiovascular risk is thought to be an anti-atherosclerotic effect, mediated by a combination of reduced plasma lipids, especially in the post-prandial setting where substantial reductions in triglycerides and ApoB48 has been documented, in combination with reduced oxidative stress and inflammation. Animal studies showed an effect to reduce atherosclerosis as well as an effect on NASH, and reduced inflammation was found in both aortic plaque tissue and liver. The GLP-1 receptor was not expressed in atherosclerotic plaques or the immediate nearby vessel wall, or in the liver, but reduced inflammation may be mediated via GLP-1 receptors in the intestine linked to reduced inflammation and improved gut barrier function. A proposed mechanism for efficacy in NASH for GLP-1 receptor agonists includes reduced inflammation, lipids, body weight and potentially also glucose lowering in patients with diabetes.

Disclosure of Interest: L. Knudsen:Stockholder: Minor employee based stockholder of Novo Nordisk, Employee: Full tiem employee of Novo Nordisk

11

Academic overview: Oxidative stress, ER stress, mitochondrial dysfunction

Massimo Pinzani* [1]

[1]*UCL Institute for liver and digestive health, Division of medicine, UCL, London, United Kingdom*

Corresponding author's email: m.pinzani@ucl.ac.uk

Non-alcoholic fatty liver (NAFL) has a prevalence of 20% in the Western world. Approximately one-third of patients with NAFL progresses in the fibrosis stage during a five-year follow-up, some of whom have a more rapid course. Liver fibrosis represents one of the key features of non-alcoholic steatohepatitis (NASH) and represents single histopathologic feature with the greatest impact on mortality. However, increasing evidence indicates that fibrosis progression is also observed in patients with NAFL, particularly in those with mild inflammatory changes, delicate fibrosis, older age or deterioration of metabolic risk factors. However, patients with NASH have a more rapid course, with a significant risk for liver-related mortality.

Because of the high incidence in the general population, a crucial step is represented by operating an initial diagnostic screening in primary care by employing algorithms, based on simple blood tests, and able to predict the presence of liver fibrosis. The following step is to identify which patients are at greatest risk for progressing to cirrhosis is essential for targeting therapeutic interventions. Indeed, several studies have demonstrated the importance of any degree of liver fibrosis in the setting of NAFLD in predicting adverse outcomes. Although liver biopsy still represents the gold standard for the diagnosis of NASH and for disease staging, the use of non-invasive methods is rapidly becoming more established and definitely useful for a rapid selection of patients to be further investigated.

The mechanisms for the transition from simple steatosis to NASH are multiple and still not yet sufficiently elucidated. Multiple hits, including genetic and epigenetic changes, fat accumulation, insulin resistance and the influence of intestinal microbiota changes seems relevant for the progression of NASH. NAFL is strongly associated with obesity, which induces adipokine secretion, endoplasmic reticulum (ER) and oxidative stress at the cellular level, which in turn induces hepatic steatosis, inflammation and fibrosis. Among these factors, gut microbiota are acknowledged as having an important role in initiating this multifactorial disease. Oxidative stress is considered to be a key contributor in the progression from NAFL to NASH. Macrophage infiltration is apparent in NAFL and NASH, while T-cell infiltration is apparent in NASH. In addition, current evidence suggests that periportal components, including the ductular reaction and expansion of the hepatic progenitor cell compartment, may be involved and that the Th17 response may mediate disease progression.

The therapeutic options in NAFLD/NASH include lifestyle modification, pharmacological treatment, and bariatric surgery for patients with morbid obesity and treatment of complications of liver cirrhosis and HCC, including liver transplantation. Insulin sensitizers and antioxidative treatment strategies with vitamin E are among the best-established pharmacological approaches, but both drugs have long-term safety issues and there is limited evidence in cirrhotic patients. Several clinical trials addressing a number of potential mechanisms responsible for NASH progression are currently ongoing.

Disclosure of Interest: None Declared

13

Academic overview: Inflammation and immunity in NAFLD: focus on macrophage heterogeneity in the liver

Frank Tacke* [1]

[1]*Dept of Medicine III, University Hospital Aachen, Aachen, Germany*

Corresponding author's email: frank.tacke@gmx.net

Non-alcoholic fatty liver disease (NAFLD) and its progressive inflammatory form, non-alcoholic steatohepatitis (NASH), are leading causes of liver cirrhosis and hepatocellular carcinoma. Metabolism and inflammation are intimately interrelated in NAFLD/NASH, as expressed by the term immunometabolism. Macrophages represent a key cellular immunological component of the liver that ensures homeostasis and rapid responses to hepatic injury. Thereby, hepatic macrophages mediate inflammatory responses during metabolic disorders and can stimulate or dismantle liver fibrosis. Their functional diversity is partly explained by heterogeneous macrophage subsets, i.e. tissue-resident Kupffer cells and monocyte-derived macrophages. However, macrophages themselves are altered in their functional polarization by dietary composition, metabolic or inflammatory stimuli in NAFLD. The inflammatory polarization of macrophages correlates with changes in core metabolism pathways like oxidative phosphorylation and glycolysis. The availability of nutrients, such as glucose or fatty acids, or oxygen also influences macrophage polarization upon danger signal or cytokine reception. Understanding the interplay of metabolism and macrophage function in NASH may open new approaches to therapeutic targeting of these essential modifiers in metabolic liver diseases. Studies are ongoing to pharmacologically inhibit inflammatory monocyte influx into the liver or to augment the differentiation of restorative macrophages. The oral dual chemokine receptor CCR2/CCR5 antagonist cenicriviroc significantly inhibits the accumulation of monocyte-derived inflammatory macrophages in animal models of liver injury, thereby ameliorating experimental steatohepatitis and hepatic fibrosis. This concept is currently being evaluated in clinical trials in patients with NASH and fibrosis.

Disclosure of Interest: F. Tacke: Grant/Research Support: Tobira/Allergan, Galapagos, Noxxon, Consultant / Advisor: Tobira/Allergan, Intercept, Galapagos, Boehringer, Sponsored Lectures: National or International: Gilead, BMS, AbbVie, Falk, Novartis, MSD

14

Inhibitors of CCR2 and CCR5

Eric Lefebvre[*1]

[1]*Allergan plc, South San Francisco, United States*

Corresponding author's email: elefebvre@tobiratx.com

Non-alcoholic fatty liver disease (NAFLD) is a common liver disorder; non-alcoholic steatohepatitis (NASH) is a more progressive phenotype associated with liver fibrosis, progression to cirrhosis and increased risk of hepatocellular carcinoma, cardiovascular disease and other non-liver cancers. C-C chemokine receptors types 2 (CCR2) and 5 (CCR5) are expressed on bone marrow-derived monocytes and macrophages, Kupffer cells and hepatic stellate cells. CCR2/5 signalling pathways have been shown to promote macrophage infiltration in response to liver injury and fibrogenesis.

Cenicriviroc (CVC) is a first-in-class, oral, dual CCR2/5 antagonist with nanomolar potency currently undergoing Phase 3 evaluation for treatment of liver fibrosis in adults with NASH (AURORA; NCT03028740). CVC has demonstrated a favourable safety and tolerability profile in over 1000 participants treated to date, including those with cirrhosis and mild to moderate hepatic impairment. CVC has shown anti-inflammatory and antifibrotic activity in multiple, well-characterised animal models of acute liver injury and fibrosis. A post hoc analysis of a Phase 2b study in HIV-1-infected participants supported the preclinical findings, showing potent CCR2/5 blockade by CVC and reductions in non-invasive markers of hepatic fibrosis (Aspartate Aminotransferase to Platelet Ratio Index [APRI] and Fibrosis-4 [FIB-4]), which were significantly correlated with decreases in soluble cluster of differentiation 14 (sCD14), a marker of monocyte activation, after 48 weeks of treatment. Together, these findings paved the way for evaluation of CVC in a Phase 2b study (CENTAUR; NCT02217475) in participants with NASH with liver fibrosis (stages 1-3, NASH Clinical Research Network [CRN] system).

In the CENTAUR Phase 2b study, the primary endpoint of improvement in NAFLD activity score (NAS) by ≥2 points (with a ≥1-point reduction in either lobular inflammation or hepatocellular ballooning) and no worsening of fibrosis stage at Year 1 (primary analysis) was met by comparable proportions of participants receiving CVC and placebo. However, twice as many participants receiving CVC vs placebo met the pre-specified key secondary endpoint of improvement in fibrosis by ≥1 stage and no worsening of NASH at Year 1, establishing proof-of-concept in adults with NASH and liver fibrosis. Treatment benefits with CVC were greater in participants with higher disease activity (i.e. NAS ≥5, prominent hepatocellular ballooning) and fibrosis stage (i.e. stages 2 and 3) at baseline, CVC treatment reduced systemic markers of inflammation, and safety and tolerability were comparable to placebo. These data support the Phase 3 development of CVC in adults with liver fibrosis (stages 2 and 3, NASH CRN) associated with NASH.

The pathophysiology of NASH involves numerous mechanisms, and several investigational agents are currently in clinical trials, allowing the potential for multitargeted therapies. CVC has undergone preclinical evaluation in combination with Novartis' farnesoid X receptor (FXR) agonist tropifexor (LJN452) in a diet-induced mouse model of NASH, which has recently demonstrated that combination therapy with CVC and LJN452 resulted in reduction of liver fibrosis relative to vehicle control animals. Findings from this study showed that combination therapy resulted in greater reductions in NAS, fat deposition, lobular inflammation and hepatocellular ballooning compared to monotherapy. A Phase 2b study of CVC and tropifexor in adults with NASH and liver fibrosis is planned. In addition, Allergan's proprietary FXR agonist AGN-242266 has recently entered Phase 1 development and combination studies with CVC are planned.

Fibrosis stage is the only histological feature of NASH independently linked to an increased likelihood of liver-related events and all-cause mortality in recent studies. Therefore, reducing liver fibrosis is expected to improve the long-term clinical outcomes of patients with NASH. Should antifibrotic benefits be corroborated by subsequent confirmatory trials, CVC will represent an important advance in the treatment of liver fibrosis in patients with NASH.

Disclosure of Interest: None Declared

15

Oral immune therapy for NASH: A new class of drugs that target the gut immune system

Yaron Ilan* [1]

[1]*Medicine, Hebrew University Hadassah Medical Center, Jerusalem, Israel*

Corresponding author's email: ilan@hadassah.org.il

Therapy for NASH: current obstacles
Much progress has been achieved in the understanding of the pathophysiology, diagnosis, and natural history of nonalcoholic steatohepatitis (NASH). However, during the process of drug development for NASH, numerous obstacles have been identified: A need for drugs which target multiple mechanisms that underlie the pathogenesis of the disease; a need for drugs with high safety profiles to enable long term therapy; and the need for targeting the whole spectrum of the disease, from steatosis to cirrhosis, are altogether hard to achieve goals.

Low-grade chronic inflammation underlines the pathogenesis of NASH
The systemic immune system plays a role in inflammation and fibrogenesis associated with NASH and has become a potential target for drug development. The metabolic inflammatory state, termed "metaflammation," is defined as a low-grade, chronic inflammation, which involves the liver, adipose tissue, pancreas, and muscle. It is associated with disruption of the metabolic homeostasis. Imbalances between pro-inflammatory and anti-inflammatory cytokines, alterations in insulin responses, β-oxidation, lipid storage and transport, Autophagy and nuclear receptor signaling, further contribute to disease progression.

Oral immune therapy for NASH: Targeting the gut immune system for alleviation of the systemic inflammatory response without immune suppression
Gut-liver crosstalk is implicated in the impairment of lipid and glucose homeostasis in steatogenesis and in the initiation of inflammation and fibrogenesis in NASH. The activation of the innate immune system in the gut, accompanied by stimulation of the systemic adaptive arm of the immune system, can lead to steatosis, liver inflammation, and fibrosis. Oral immunotherapy is based on the notion that targeting various components of the gut barrier can serve as a means to alter the immune signal that is transported from the gut to the systemic immune system, thereby affecting immune-mediated damage in target organs. DCs are central for the initiation and differentiation of adaptive immune responses. The mesenteric lymph nodes (MLNs) serve as a site for the generation of these immune signals. Oral immunotherapy compounds that target the gut-associated innate immune system and/or Microbiome, generate an immune signal that induces regulatory T cells (Tregs).

A new class of drugs for NASH: pre-clinical and clinical studies
Several methods have recently been investigated, targeting the gut immune system to generate anti-inflammatory signals that redirect the systemic immune system. Some of these methods have been demonstrated to be effective in pre-clinical trials, and some were shown to exert beneficial responses in phase I and II clinical trials in patients with NASH. These include oral administration of fatty-liver-derived proteins; oral administration of anti-CD3 antibodies; oral anti-tumor necrosis factor (TNF) fusion protein; anti-lipopolysaccharide (LPS) antibodies; oral administration of beta-Glucosylceramide; oral use of delayed-release Mercaptopurine; and oral soy-derived extracts. All of these compounds exert an effect at the level of the gut immune system.

The process of an oral administration and lack of absorption make these compounds easy to use and well tolerated. An Oral administration of these compounds provides an opportunity for immune modulation without immune suppression, with the advantage of being independent of a single molecular/inflammatory pathway. Oral immune therapy has the advantage of being "antigen- and molecular pathway-independent." Consequently, these compounds may provide broader "coverage" of the inflammatory pathways relevant to NASH.

Oral immune therapy compounds show potential for use in patients with all degrees of disease severity. It is likely that these oral compounds can be used to induce remission as well as long-term maintenance. They provide a platform for the treatment of concomitant disorders in patients with NASH, including diabetes and hyperlipidemia. Because no absorption is required and a relatively low dose is sufficient to achieve a clinically meaningful effect, these compounds are expected to be relatively affordable for long-term use. It is anticipated that the acquisition of more clinical data in the next few years will enable the use of this new class of drugs for the treatment of NASH.

References

[1] Ilan Y. Novel methods for the treatment of nonalcoholic steatohepatitis - targeting the gut immune system to decrease the systemic inflammatory response without immune suppression. Aliment Pharmacol Ther. 2016 Dec;44(11-12:1168-1182

Disclosure of Interest: Y. Ilan: Sponsored Lectures: National or International: Consultant: Teva; Abbott; ENZO; Protalix; Therapix; Immunepharma; JTI; Immuron; Nasvax; Alcobra; Accelmed; Medial; SciM; Medical Director:Natural Shield; Oberon Sciences; Tiziana Pharma; Plantylight; Exalenz Bioscience;

16

Role of inflammasomes in NAFLD

Fabio Marra[*1]

[1]*Dipartimento di Medicina Sperimentale e Clinica, Università degli Studi di Firenze, Florence, Italy*

Corresponding author's email: fabio.marra@unifi.it

Toll-like receptors (TLRs) are highly conserved receptors that recognize endogenous danger signals, such as molecules released by damaged cells (damage-associated molecular patterns, DAMPs) or exogenous danger signals, as gut-derived pathogen-associated molecules (pathogen-associated molecular patterns, PAMPs). As pattern recognition receptors (PRR), TLRs act as defense mechanism, but are also implicated in the pathogenesis of NASH. An important role in NASH pathogenesis has been recently ascribed to the nucleotide oligomerization domain (NOD)-like receptors (NLRs). NLR activation in response to DAMPs or PAMPs, leads to assembly of inflammasome, a multiprotein complex required for caspase-1 activity and consequent initiation of inflammatory signals. Full activation of inflammasome is induced via NF-κB by a broad spectrum of signals, such as PRR activation, uric acid, ROS, ATP and mitochondrial DNA. Inflammasome activation results in increased secretion of mature IL-1 and IL-18. These cytokines, acting on different cell types, elicit inflammatory signals in the liver as well as in the adipose tissue and intestine, mediating steatosis, insulin resistance, inflammation and cell death. A role for inflammasomes in NAFLD development and progression to NASH has been shown both in humans and animal models. Activation of NLRP3 inflammasome has been reported in steatohepatitis induced by the MCD diet, as well following feeding with a high fat, high-cholesterol, high sucrose diet. In addition, models characterized by a NLRP3 gain of function are characterized by enhanced fibrosis. Conversely, absence of this receptor appears to improve metabolic activity and diet-induced steatohepatitis, although in another study, it has been shown that lack of NLP3 promotes gut dysbiosis and chronic inflammation. This indicated that activation of this system at different levels or in different cell type may result in different phenotypes. Activation of NLRP3 inflammasome has been associated with hepatocyte pyroptosis, a recently described, inflammasome-mediated, cell death mechanism. One of the relevant mechanisms underlying inflammasome activation is represented by release of DAMPS by damaged hepatocytes. These molecules act as danger signals capable to recruit and/or activate immune cells and initiate an inflammatory response in the absence of pathogens, a mechanism referred as sterile inflammation. Several DAMPs have been identified, including nuclear and mitochondrial DNA, purine nucleotides (ATP, UTP), nuclear factors as high-mobility group box 1 (HMGB1) and uric acid. Besides mitochondrial DNA, which activates TLR9, a number of mitochondrial components have been shown to play a part in sterile inflammation, including formyl-peptides, ATP and ROS that also act inducing inflammasome activation. High concentrations of extracellular ATP, as a consequence of cell death, result in inflammasome activation and IL-1β production, via P2X7 receptor. As binding of ATP to P2X7 provokes pore formation in the plasma membrane, allowing bacterial products to enter the cells, ATP plays a role also in pathogen-associated molecular pattern-induced inflammation. HMGB1 is a constitutively expressed nuclear protein that is released in response to different stimuli, such as PAMPs and DAMPs. HMGB1 interacts with a broad spectrum of receptors exerting proinflammatory actions. In some settings, DAMPs can be also secreted independently of apoptosis. HMGB1 production can occur by activated macrophages in response to LPS, TNF, and TGFβ. Activation of the inflammasome system in stellate cells has been implicated in the pathogenesis of fibrosis.

Different types of inflammasomes exist and the role of some of these systems is not yet completely understood. Nonetheless, NLRP3 inflammasome currently represents an attractive target for further investigation of therapeutic strategies in NASH.

Disclosure of Interest: None Declared

17

VAP-1 inhibitors: Role of AOC3/VAP-1/SSAO in liver inflammation and small

Joerg F. Rippmann[*1], Jörg Hehn[2], Alexander Pautshe[2], Oliver Hucke[3], Brigit Stierstorfer[4], Andre Broermann[1]

[1]CardioMetabolic Diseases Research, [2]Medicinal Chemistry Department, [3]Medicinal Chemistry Department, [4]Target Discovery Research Department, Boehringer Ingelheim Pharma Gmbh & Co. Kg, Biberach an der Riss, Germany

Corresponding author's email: joerg.rippmann@boehringer-ingelheim.com

---

The transmembrane protein AOC3 (amine-oxidase copper containing 3), also known as the vascular adhesion protein-1 (VAP-1) or the semicarbazide sensitive amine oxidase (SSAO) is mainly expressed in adipocytes, smooth muscle cells and myofibroblasts. The expression of AOC3 in normal human liver will be shown, which is restricted to sinusoidal endothelial cells. AOC3 expression extends towards fibrotic areas under NASH conditions probably due to the engagement of newly derived myofibroblasts and the activation of endothelial cells. The biological function of AOC3 was described by genetic depletion or inhibition experiments as a leukocyte recruitment factor and a pro-inflammatory peroxide generator. AOC3 is also shed from the cellular membrane and the activity in plasma can serve as a biomarker for drug development. The extracellular domain of AOC3 crystallizes as a dimer including two copper ions as essential elements of the enzymatic function. Several structural classes of small molecule inhibitors have been described and their binding and inhibition mode will be show in a model structure of AOC3. Inhibition of AOC3 with a representative molecule of the fluoroallylamines class in a diet induced NASH model and the consequence on inflammation and fibrotic readouts will be shown.

Disclosure of Interest: J. Rippmann: Employee: Rippmann Boehringer Ingelheim Pharma GmbH Co KG, J. Hehn: Employee: Rippmann Boehringer Ingelheim Pharma GmbH Co KG, A. Pautshe: Employee: Rippmann Boehringer Ingelheim Pharma GmbH Co KG, O. Hucke: Employee: Rippmann Boehringer Ingelheim Pharma GmbH Co KG, B. Stierstorfer: Employee: Rippmann Boehringer Ingelheim Pharma GmbH Co KG, A. Broermann: Employee: Rippmann Boehringer Ingelheim Pharma GmbH Co KG

18

Academic overview: Apoptosis and microparticles

Ali Canbay* [1]

[1]*Universitätsklinikum Magdeburg, Magdeburg, Germany*

Corresponding author's email: ali.canbay@uni-due.de

Apoptosis in NAFLD – background
Apoptosis was discovered as fundamental process during morphogenesis and development of tissues. Beyond its role in organism development, apoptosis is possibly the most important type of regulated cell death in liver tissue under conditions of liver injury. In hepatocytes, mitochondrial outer membrane perforation is usually required to initiate apoptosis, even for extrinsic signals leading to apoptosis. The release of cytochrome c from mitochondria promotes formation of the apoptosome and subsequent activation of the effector caspases 3, 6 and 7. In human non-alcoholic steatohepatitis (NASH) hepatocellular apoptosis has been found and expression of death ligands and death receptors is increased in liver tissue of NASH patients.In most human studies the focus was on morphological identification of cell death patterns, which may be misleading, as they are not absolutely specific. Biochemical classification of cell death is preferable, but rarely possible in the human situation. For other cell death modes in non-alcholic fatty liver disease (NAFLD), clinical data are either not available or conflicting. For pyroptosis and necroptosis pre-clinical data suggest a possible role in the progress from NAFL to NASH, which needs to be confirmed.

Apoptosis as biomarker
Since detection of in situ cell death in humans is difficult or sometimes impossible, surrogate markers for apoptosis are in use. Severity of the injury can be indicated by M30 (an antibody combination), which can detect caspase-cleaved cytokeratin 18 (CK-18). CK-18 is an epithelial cell marker and thus not specific for liver disease. Though, in established liver dieses M30 has shown consistent correlation to severity of liver injury, including severity of NAFLD. Serum concentrations of caspase 3 seem to be independent of severity of liver injury. For detection of pyroptosis or necroptosis IL-1, IL-18 or HMGB1, respectively, could be employed. However, no or only insufficient clinical studies exist on these markers, yet.

Apoptosis as therapeutical target
Despite pre-clinical evidence for caspase inhibitors as possible therapy for NASH, clinical trials were not able to demonstrate sufficient efficacy for approval in NAFLD or NASH. This might be due to a mere switch from one type of cell death to another – unknown – cell death mode. Also it might be possible that stopping apoptosis in ongoing chronic liver injury occurred too late with too little impact on the liver damage. Moreover safety concerns regarding tumorigenicity of apoptosis inhibitors have not been cleared, yet. One interesting pre-clinical observation, partially confirmed in human samples is a switch from caspase-8-dependent apoptosis to necroptosis during the progression to NASH. Thus, the cellular components inhibiting caspase-8, would be an interesting target to counter progression by reconstituting caspase-8-dependent apoptosis.

Microparticles in NAFLD - background
Cells release particles in a variety of sizes and manners. Microparticles (MPs) are small membrane-bound particles released by membrane shedding (ectocytosis) in a process that involves a regulated sorting of membrane proteins into the membrane of the MP. Also phosphatidylserine is flipped from the inner to the outer membrane in MPs during cellular activation or early apoptosis (apoptotic bodies) MPs have a diameter of 100 - 1000 nm in size (exosomes are only 30-100 nm). MP resemble their parental cells on a smaller scale and share with them many characteristics, such as surface receptors, integral membrane, cytosolic and nuclear proteins, RNAs including miRNAs. By

transporting this variety of molecules MP facilitate cell-to-cell communication, with specific roles depending on the cell type releasing the MP and the composition. In patients with NAFLD increased numbers of microvesicles derived from macrophages and natural killer T cells have been observed.

Microparticles as biomarker
In NAFLD macrophage and NKT-cell specific MP were elevated. Since both cell types are probably involved in the inflammatory component of NASH, these specific MP might be associated to the severity of NAFLD. Further studies are warranted to support this hypothesis and to show better performance than other inflammatory markers.Application of microparticles or targeting microparticles as possible therapeutic option is a promising idea to reduce inflammatory components of NAFLD/NASH. Nevertheless the scientific basis and evidence for such an approach is currently lacking. In particular most studies on MP derive from preclinical models. Studies in human populations – healthy and diseased – are needed to generate basic knowledge in this area.

Disclosure of Interest: None Declared

20

Extracellular vesicles as NASH biomarkers

Ariel Feldstein* [1]

[1]*Pediatrics, UCSD, San Diego, United States*

Corresponding author's email: afeldstein@ucsd.edu

Liver biopsy remains the gold standard procedure for diagnosis and staging of NAFLD. While various imaging modalities are growingly been used for assessment of liver fibrosis, these techniques lack sensitivity and specificity for early stages of fibrosis and are not useful for determination of inflammation and hepatocellular injury. Based on the growing evidence for a key pathophysiological role of extracellular vesicles (EVs), small membrane bound vesicles that are released in a stress-specific manner and play a central role in cell to cell communication, in liver injury in conjunction with the fact that EVs are released into various bodily fluids and are remarkable stable make them potential ideal targets for biomarkers development. Different approaches have been used including analyzing changes in EV types and cell-specific derived EVs by using selective surface markers or EV cargo as well as untargeted comprehensive approaches to assess EV composition such as protein, lipids, or RNA. In this talk, we will discuss the current early evidence pointing to a potential significant role of assessing EVs and EV composition for novel biomarkers development and the key future studies that are needed to further interrogate and validate these novel approaches.

Disclosure of Interest: None Declared

21

Keratins as biomarkers

Heike Bantel* [1]

[1]*Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany*

Corresponding author's email: bantel.heike@mh-hannover.de

Keratins represent the major epithelial-specific subgroup of intermediate filament proteins and can be divided into type I (K9-28, K31-40) and type II (K1-8, K71-86) keratins [1]. Epithelial cells express at least one type I and one type II keratin in a cell-type specific manner. For instance, adult hepatocytes express K8 and K18 which function primarily to maintain hepatocyte integrity. In liver diseases, however, increased hepatocyte apoptosis leads to cytoskeletal disintegration and liver injury. During apoptosis of hepatocytes, caspases become activated and cleave K18 at a typical aspartate cleavage sequence thereby exposing an epitope which can be recognized by the M30 antibody [2]. This antibody therefore allows for the detection of hepatocyte apoptosis in liver tissues. Moreover, caspase-generated K18-fragments are released from apoptotic hepatocytes and can be non-invasively detected in sera from patients with liver diseases by the M30 enzyme-linked immunosorbent assay (ELISA) [3]. Because hepatocyte apoptosis plays a critical role in the pathogenesis and progression of non-alcoholic fatty liver disease (NAFLD) [4], and aminotransferases are not sensitive enough for the diagnosis of non-alcoholic steatohepatitis (NASH) [5], the M30 ELISA became an extensively evaluated biomarker for detection of NAFLD activity. In this context, it has been demonstrated that patients with NASH reveal higher plasma/serum levels of caspase-generated K18 compared to patients with simple steatosis [6-8]. K18 fragments significantly correlated with histological components of the NAFLD activity score (NAS), i.e. the degree of steatosis, inflammation and ballooning, and more accurately reflected NAS compared to aminotransferase levels [9]. There is also increasing evidence that the M65 ELISA, which detects caspase-cleaved and uncleaved (total) K18, might be suitable for monitoring NAFLD activity. Moreover, this assay was able to predict NASH independently of ALT levels [10] which might be important, since NASH can be histologically detected in more than 50% of NAFLD patients despite normal aminotransferase levels [5]. Taken together, caspase-cleaved and total K18 might represent reliable biomarkers for detection and monitoring of NAFLD activity and are increasingly used in prospective NAFLD studies, contributing to our understanding of their diagnostic performance in clinical practice.

References:

[1] Ku N-O, Strnad P, Bantel H, Omary MB. Keratins: biomarkers and modulators of apoptotic and necrotic cell death in the liver. Hepatology 2016; 64:966-76.
[2] Bantel H, Ruck P, Gregor M, Schulze-Osthoff K. Detection of elevated caspase activation and early apoptosis in liver diseases. Eur J Cell Biol 2001; 80:230-39.
[3] Bantel H, Legering A, Heidemann J, Volkmann X, Poremba C, Strassburg CP, Manns MP, Schulze-Osthoff K. Detection of apoptotic caspase activation in sera from patients with chronic HCV infection is associated with fibrotic liver injury. Hepatology 2004; 40:1078-87.
[4] Syn WK, Choi SS, Diehl AM. Apoptosis and cytokines in non-alcoholic steatohepatitis. Clin Liver Dis 2009; 13:565-80.
[5] Fracanzani AL, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, Bertelli C, Fatta E, Bignamini D, Marchesini G, Fargion S. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. Hepatology 2008; 48:792-8.

[6] Feldstein AE, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. Hepatology 2009; 50:1072-8.
[7] Bantel H, John K, Schulze-Osthoff K. Robust detection of liver steatosis and staging of NAFLD by an improved ELISA for serum cytokeratin-18 fragments. Am J Gastroenterol 2014; 109:140-1
[8] Bechmann LP, Kocabayoglu P, Sowa JP, Sydor S, Best J, Schlattjan M, Beilfuss A, Schmitt J, Hannivoort RA, Kilicarslan A, Rust C, Berr F, Tschopp O, Gerken G, Friedman SL, Geier A, Canbay A. Free fatty acids repress small heterodimer partner (SHP) activation and adiponectin counteracts bile acid-induced liver injury in superobese patients with nonalcoholic steatohepatitis. Hepatology 2013; 57:1394-406.
[9] Tsutsui M, Tanaka N, Kawakubo M, Sheena Y, Horiuchi A, Komatsu M, Nagaya T, Joshita S, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Aoyama T, Tanaka E, Sano K. Serum fragmented cytokeratin 18 levels reflect the histologic activity score of nonalcoholic fatty liver disease more accurately than serum alanine aminotransferase levels. J Clin Gastroenterol 2010; 44:440-7.
[10] Joka D, Wahl K, Moeller S, Schlue J, Vaske B, Bahr MJ, Manns MP, Schulze-Osthoff K, Bantel H. Prospective biopsy-controlled evaluation of cell death biomarkers for prediction of liver fibrosis and nonalcoholic steatohepatitis. Hepatology 2012; 55:455-64.

Disclosure of Interest: None Declared

22

Academic overview: Therapeutic and prognostic relevance of bile acids and bile acid signaling in NASH

Michael Trauner* [1]

[1]*Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Austria, Vienna, Austria*

Corresponding author's email: michael.trauner@meduniwien.ac.at

Besides their well-established roles as detergents for lipid digestion/absorption and cholesterol homeostasis, bile acids (BA) also act as metabolically active signalling molecules. The flux of reabsorbed BA undergoing an enterohepatic circulation, arriving in the liver with the co-absorbed nutrients (e.g. glucose, lipids) provides a signal that coordinates hepatic triglyceride (TG), glucose and energy homeostasis. In addition, BA interact with gut microbiota and are important gatekeepers of intestinal integrity. Moreover, BAs have important anti-inflammatory and immunomodulatory actions, control gut integrity/permeability and modulate gut microbiota with broader implications for the gut-liver axis in liver diseases. Activation or modulation of BA receptors, such as the farnesoid X receptor (FXR) and TGR5, and transporters such as the ileal apical sodium-dependent bile acid transporter (ASBT) may affect NAFLD/NASH pathogenesis at multiple levels, and these approaches hold promise as novel therapies. Additional targets for BA signaling include other BA-activated NRs (vitamin D receptor, VDR; pregnane X receptor, PXR) and other BA-activated receptors (e.g., Sphingosine-1-Phosphate Receptor 2, muscarinic receptors M2 & M3).

FXR and its downstream targets such as fibroblast growth factor (FGF) 19 play a central role in the control of hepatic lipid and glucose homeostasis. BA-activated FXR and signal transduction pathways are involved in the regulation of hepatic de novo lipogenesis, very low density lipoprotein (VLDL)-TG export and plasma TG turnover, as well as hepatic gluconeogenesis, glycogen synthesis and insulin sensitivity. FXR-induced FGF-19 regulates lipogenesis, (indirectly) FA-oxidation and glycogenesis. Via TGR5 BA are also able to stimulate glucagon-like peptide-1 (GLP-1) secretion in the small intestine and energy expenditure in brown adipose tissue and skeletal muscle. Dysregulation of BA transport and impaired BA receptor signalling may contribute to the pathogenesis of NAFLD. Recently genetic and acquired alterations of FXR in NAGFLD/NASH have receivced considerable attention as potential ratioanle for FXR-directed therapies. Serum and fecal BA profiles have been linked to the severity of NASH and may serve as a biomarker in NAFLD, although these changes could also reflect underlying insulin resistance. In addition, recent developments have focused on the role of FXR for the treatment of portal hypertension in NASH cirrhosis through modulation of sinusoidal endothelial cell function and hepatic stellate cell contractility as major determinants of intrahepatic resistance.

Therefore, BA transport and BA-controlled nuclear receptors and signalling pathways are promising drug targets for treatment of NAFLD/NASH. As such FXR and/or TGR5 ligands have shown promising results in animal models and clinical pilot studies in NAFLD/NASH. The first in class steroidal FXR ligand obeticholic acid (OCA) improved liver enzymes and histology in NASH, but its long-term benefits and safety need further clarification. In a pilot study, the non-steroidal FXR agonist PX-104 improved insulin sensitivity and serum liver enzymes in non-diabetic NAFLD patients. Several non-steroidal FXR ligands and FGF-19 mimetics are currently undergoing phase II trials in NAFLD/NASH. Modulation of BA transport within the enterohepatic circulation (via ASBT, NTCP and BSEP) may also modify BA signaling along the gut-liver axis. ASBT inhibitors have shown interesting results in preclinical models thorugh alterations in cholesterol homeostasis and shifting BA composition/signaling towards FXR agonisms. Despite being a poor FXR and TGR5 ligand with even some FXR antagonistic actions in vivo, ursodeoxycholic acid (UDCA) improves hepatic ER stress and

insulin sensitivity in patients with NAFLD. Notably, norUDCA, a side chain-shortened homologue of UDCA, improves fatty liver/NASH and atherosclerosis in preclinical mouse model and has recently shown pormising results in a pilot phase II study in NAFLD. Collectively, these findings suggest that BA and targeting their receptors/signalling pathways may represent a promising approach to treat NAFLD/NASH and closely linked disorders such as obesity, diabetes, dyslipidemia and arteriosclerosis.

Disclosure of Interest: None Declared

24

Steroidal FXR agonists in the treatment of liver and metabolic diseases

Luciano Adorini* [1]

[1]*Intercept Pharmaceuticals, Milan, Italy*

Corresponding author's email: ladorini@interceptpharma.com

The farnesoid X receptor (FXR) is a nuclear receptor primarily expressed in the liver, gut and kidney that recognizes endogenous bile acids (BAs). Ligand-dependent FXR activation coordinately regulates BA, lipid and glucose metabolism. In addition, FXR activation protects against cholestatic and fibrotic liver damage, promotes liver regeneration, controls intestinal bacterial growth, and inhibits inflammatory processes. FXR is also involved at multiple steps in insulin signaling pathways, indicating its potential in the treatment of liver and metabolic disorders.

With the search for the ideal FXR agonist as a therapeutic agent actively ongoing, different approaches to optimize their safety and efficacy are being explored. FXR agonists can be broadly categorized as steroidal or nonsteroidal compounds. Steroidal FXR agonists are typically based on the BA scaffold, in which subtle modifications can markedly change not only FXR and/or TGR5 agonist activities but also physicochemical, ADME and PK properties.

Obeticholic acid (OCA, 6α-ethyl-chenodeoxycholic acid), the prototypic FXR steroidal agonist, is a semi-synthetic derivative of the primary human bile acid chenodeoxycholic acid (CDCA), and is approximately 100 times more potent than CDCA as an FXR agonist (EC_{50} ~100 nMol). OCA has been evaluated in different models of acute and chronic liver injury, demonstrating the capacity to stimulate bile flow and ameliorate cholestasis, to inhibit hepatic inflammation, and to reverse fibrosis, cirrhosis, and portal hypertension. OCA is also effective in ameliorating a variety of chronic inflammatory and metabolic diseases in other FXR-expressing organs, including gut, kidney, and lung. In the gut, OCA restores the intestinal barrier, inhibits bacterial translocation and modulates the microbiota.

Clinical pharmacology Phase 1 studies have demonstrated that orally administered OCA is rapidly absorbed, exhibits secondary peaks indicating enterohepatic recirculation, and does not cause adverse events up to 100 mg/day. Based on these results, double-blind, placebo-controlled Phase 2 and Phase 3 studies have successfully tested OCA in patients with primary biliary cholangitis (PBC), leading to its approval for this indication. Additional clinical studies have also shown the capacity of OCA to improve insulin sensitivity in diabetic patients with nonalcoholic fatty liver disease (NAFLD) and to revert liver inflammation and fibrosis in nonalcoholic steatohepatitis (NASH) patients, fully confirming the preclinical data.
Thus, the steroidal FXR agonist OCA has shown clear clinical efficacy with a broad therapeutic window and manageable side effects. This has prompted the development of other BA-derived compounds, not only specific for FXR but also for TGR5, like INT-777, or with dual FXR/TGR5 agonistic activity, like INT-767. Recent work has demonstrated that the introduction of a hydroxy group at the C11β position of OCA affords exquisite specificity for FXR, without any activity on other nuclear receptors or on TGR5. Remarkably, the presence of C11β-OH not only imparts FXR specificity but also renders this OCA derivative highly water-soluble (16-fold higher than OCA), comparable to cholic acid, with a high critical micellar concentration like ursodeoxycholic acid (UDCA), which accounts for a low liability for detergency and toxicity. Thus, the OCA derivative, 3α,7α,11β-trihydroxy-6α-ethyl-5β-cholan-24-oic acid (TC-100) shows a remarkable physicochemical and pharmacological profile, combining the excellent physicochemical properties of hydrophilic BAs such

as UDCA with the distinct ability to specifically activate FXR activity in vivo, providing a potential novel therapeutic agent to treat a variety of disease, including enterohepatic disorders such as cholestasis, NASH, and inflammatory bowel disease.

All these semi-synthetic BA derivatives behave like natural BAs in terms of metabolism and enterohepatic recirculation, with predictable pharmacokinetics and safety profiles in preclinical models and in patient populations. Conversely, the non-steroidal FXR agonists being developed typically possess higher potency in terms of FXR activation, usually display a systemic distribution, and are reportedly devoid of side effects considered related to BAs, like pruritus and modulation of the lipid profile. However, these may represent FXR-mediated class effects and are already being observed with some non-steroidal FXR agonists. It remains to be seen how effectively and safely these non-steroidal FXR agonists will translate into the clinic.

Disclosure of Interest: L. Adorini: Consultant / Advisor: Intercept Pharmaceuticals

25

FXR - non-steroidal agonists

Nikolai V. Naoumov [*1]

[1]*Novartis Pharma, Novartis Global Drug Development, Basel, Switzerland*

Corresponding author's email: nikolai.naoumov@novartis.com

Farnesoid X-activated receptor (FXR) is a nuclear receptor transcription factor, originally named because of suspected ability to bind farnesoid. Bile Acids (BA) are the natural ligands for FXR through which BA regulate their own homeostasis. FXR activation leads to inhibition of BA synthesis, reduction of hepatic uptake, and increase of BA hepatocellular export. FXR is also a master regulator of lipid and glucose homeostasis, of inflammatory and fibrogenic processes.[1]

In patients with NASH, FXR agonism provides multimodal benefits: reduces fat accumulation in liver, reduces hepatic inflammation, anti-fibrotic effect, reduces bacterial translocation from gut.[2] BA metabolism is significantly altered in NASH.[3] Thus, FXR agonists are expected to have beneficial effects in NASH also through their impact on BA metabolism.

Obeticholic acid (OCA) is a derivative of the natural ligand – chenodeoxicholic acid[4] and currently the most advanced FXR agonist in development. Clinical studies with OCA have validated FXR as an important target, and demonstrated the clinical potential of FXR agonists in treatment of a wide range of liver diseases. The experience with BA-derived FXR agonists also revealed a number of limitations, many of which related to their structure. Steroidal FXR agonists usually have poor aqueous solubility and bioavailability; they undergo enterohepatic recirculation; the steroidal structure along with TGR5 activity are potential mechanisms for clinically significant and dose-limiting pruritus; there are undesirable changes in cholesterol metabolism.[5]

The great clinical potential of FXR agonism and the limitations associated with BA-derived agonists, have driven the development of synthetic non-steroidal FXR agonists. **GW4064** is the first non-steroidal FXR agonist, published in 2000[6], which was used as a "chemical tool" for testing pharmacological effects in vitro and in animal models (Figure). It never entered the clinic due to poor bioavailability, photoliability and potential toxicity.[7] Amongst the early non-steroidal agonists, **WAY-362450** is structurally independent from GW4064, was taken into Phase 1 but further development was abandoned. **Fexaramine** is another compound, published in 2003, found to have 100-fold greater affinity for FXR than natural compounds, but animal experiments revealed that the FXR agonism was limited to the intestine.[7]

Specific features of non-steroidal FXR agonists include: i) Non-Bile Acid chemical structure; ii) No enterohepatic recirculation; iii) Potent FXR activation and low systemic exposure; iv) No TGR5 activity; v) Pruritus is not expected; vi) No LDL-Cholesterol elevation. In preclinical models, some non-steroidal FXR agonists have shown significantly greater anti-fibrotic effects than OCA. For example, in the ANIT model with **LJN452** [8] and similarly in CCl_4 cirrhosis model with **PX20606 (Px102)**
A short outline of non-steroidal FXR agonists currently in development is shown below.

Phase 2:

LJN452 (tropifexor) - Novartis. PK, safety and tolerability data from First-in-Human study including single doses up to 3 mg and MAD were safe and well-tolerated.[10] Ph2 for NASH is ongoing (CT.gov NCT02855164).

GS-9674 (Gilead) is closely related to PX20606 (Px102) which was developed by Phenex.[7] Promising Ph1 results with PK and safety were presented at AASLD 2016.[11,12] Ph2 study with 140 NASH patients is ongoing (NCT02854605).

LMB763 (Novartis). A non-bile acid, potent, selective agonist with promising Ph1 results.[13] Ph2 study in NASH is ongoing (NCT02913105).
Phase 1:

EDP305 (Enanta) non-bile acid FXR agonist containing steroidal and non-steroidal components and no carboxylic acid.[14] Preclinical profiling presented at EASL 2017, with EDP305 now in Ph1. Another agonist, **EP-024297** (Enanta) showed greater potency and selectivity than OCA in preclinical tests.[15]

EYP001 (ENYO) – Ph1 in healthy subjects completed (NCT03110276). EYP001 was safe; FGF19 and C4 changes were consistent with FXR agonism. In vitro, EYP001 has shown activity against HBV replication and HBsAg.[16]

Preclinical stage:

M-480 (Metacrine). This non-bile acid FXR agonist recently showed histological improvement in animal model of NASH.[17]

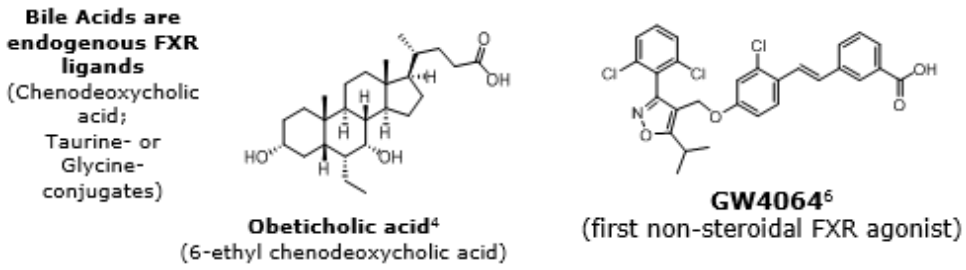
AKN083/AGN-242266 (Allergan)-a non-bile acid FXR agonist, in preclinical studies has shown high affinity, potency good tolerability.[18]

References:

[1] Nat Rev Drug Discov 2016;15:249-274;
[2] Nat Rev Gastroenterol Hepatol. 2014;11:55-67;
[3] Hepatology 2017 (in press). doi: 10.1002/hep.29359;
[4] J Med Chem 2002;45:3569-3572;
[5] J Hepatol 2017;66:680-681;
[6] J Med Chem 2000:43:2971-2974;
[7] Curr Top Med Chem 2014;14:2143-2158;
[8] APASL 2016, Kyoto, Poster 11078;
[9] J Hepatol 2017;66:724-733;
[10] Hepatology 2016;64(Suppl):16A;
[11] AASLD 2016, Boston; Poster 1077;
[12] AASLD 2016, Poster 1140;
[13] EASL 2017 Amsterdam, Poster FRI-339;
[14] Enanta, Press Release 19 April 2017;
[15] AASLD 2016, Poster 1569;
[16] J Hepatol 2017;66(Suppl): S690;
[17] EASL 2017, poster FRI-361;
[18] Allergan, Press Release Sept 2016.

Figure:

Natural ligands, steroidal and non-steroidal FXR agonists



Disclosure of Interest: N. Naoumov: Employee Novartis

26

FGF19: a downstream FXR target and an inspiration for new therapeutics in the context of NAFLD

Matias A Avila[*1]

[1]*Hepatology, CIMA-University of Navarra. CIBERehd, IdisNa, Pamplona, Spain*

Corresponding author's email: maavila@unav.es

The liver displays a remarkable regenerative response after injury or resection that is essential to guarantee survival. Understanding the fundamental components of this response may allow the development of hepatoprotective and pro-regenerative therapeutic strategies in acute and chronic liver injury. Liver regeneration has been extensively studied over the past century using experimental models of partial liver resection and chemical liver injury, with clinical findings confirming its conservation in humans. The exact nature of the signals and mechanisms involved in this process is not completely known. Most of the molecules involved in liver regeneration fall into three categories according to their chemical nature and biological properties, comprising cytokines, growh factors and metabolites. Being the liver a central metabolic organ it is conceivable that fluctuations in the levels of certain metabolites could influence, *i.e.* trigger and stop, liver growth upon damage or resection, and also maintain a liver size that meets the body needs. Among these metabolites bile acids (BAs) have attracted much attention in the past decade. This emerging role for BAs may be related to their activity as signaling molecules, capable of binding and activating nuclear receptors such as farnesoid X receptor (FXR), or the cell surface G-protein coupled receptor TGR5. One of the target genes of FXR is fibroblast growth factor 19 (FGF19, Fgf15 in rodents). FGF19 expression is triggered in ileal enterocytes during the enterohepatic circulation of BAs, and reaches the liver through the portal blood supply. FGF19 markedly regulates glucose, lipid and protein metabolism in hepatocytes, and potently inhibitis BA synthesis. FGF19 has also been identified as an important mediator of the liver regenerative response. The metabolic activities of FGF19 together with its pro-mitogenic properties, identify FGF19 an excellent candidate for development as a pro-regenerative drug, particularly in the context of cholestatic and fatty liver regeneration. In chronic liver injury associated with fatty liver disease and cholestasis FGF19-based therapy may also be of interest. However, for this purpose the pro-mitogenic activity of this factor needs to be avoided, as chronic exposure to high FGF19 levels can promote hepatocarcinogenesis. In this presentation we will review the current evidences linking BAs to the regulation of the liver regenerative response and the role of FGF19 in this process. We will also discuss the potential of engineered FGF19-based molecules as stimulators of liver regeneration and hepatoprotectants in different clinical contexts.

Disclosure of Interest: None Declared

27

ASBT inhibitors

Hanns-Ulrich Marschall* [1]

[1]*Department of Molecular and Clinical Medicine, Sahlgrenska Academy, Institute of Medicine, Gothenburg, Sweden*

Corresponding author's email: hanns-ulrich.marschall@gu.se

The apical sodium-dependent bile salt transport (ASBT), also called ileal bile acid transporter (IBAT, SLC10A2), is a key element in the enterohepatic circulation of bile acids [1]. It is an integral brush border membrane glycoprotein mainly expressed in the distal ileum and responsible for the reabsorption of about 90-95% of the intestinal bile acids, predominantly in the glycine- or taurine-conjugated forms, that are then recirculated to the liver via portal venous blood. Depleting the bile acid pool by intestinal ASBT inhibition may emerge as an option for the treatment of NASH and cholestatic liver disease [2]. So far, the majority of preclinical and clinical trials have addressed the effect of intestinal ASBT inhibition in cholestatic liver disease.

Studies with ASBT inhibitors SC-435 [3] and A4250 [4], respectively, in two independent studies with Mdr2 knock-out mice that are established models of sclerosing cholangitis consistently demonstrated improvements of cholestatic liver and bile duct injury related to reduction of bile acid pool size.

In a randomised double-blind, single-ascending dose and multiple-ascending-dose study including 40 individuals in total, A4250 decreased ileal FXR-dependent FGF19 secretion, which resulted in increased bile acid synthesis as estimated by the synthesis marker alpha-hydroxy-4-cholesten-3-one (C4). Plasma bile acid decreased consistent with increased faecal bile acid excretion [5]. Treatment with A4250 was not associated with adverse events other than those associated with the mechanism of action of an ileal ASBT inhibitor, i.e. bile acid-induced increase in the number of bowel movements [5].

So far, three clinical phase IIa trials with intestinal ASBT inhibition for the treatment of cholestatic pruritus in patients with primary biliary cholangitis have been performed of which only the second is published as full paper. Maralixibat (Lopixibat) was administered for 12 weeks in 66 PBC patients. This ASBT inhibitor was not more efficient on pruritus than placebo, however, serum bile acids decreased and C4 increased, and a higher number of diarrhoea and abdominal pain was reported, compared to placebo [6].

The ASBT inhibitor GSK2330672 was tested in 22 PBC patients for 2 weeks. This drug significantly alleviated itch as estimated by VAS, PBC-40 itch domain and 5-D itch scale and also reduced serum bile acids and FGF19 while C4 increased [7]. Of note, 33% of the patients administered GSK2330672 reported diarrhoea and a substantial number of patients reported abdominal pain [7].

In our pilot study in 10 patients with PBC that were on previous bile salt sequestrants therapy, four weeks administration of A4250 resulted in beneficial effects on pruritus, again as estimated by VAS, PBC-40 itch domain and 5-D itch scale. However, 5 patients prematurely discontinued due to abdominal side effects [8]. Of note, in children with various cholestatic liver diseases, alleviation of pruritus by A4250 was not complicated by diarrhoea or abdominal complaints [9].

In high-fat-diet-fed mice as a mouse model of NASH, ASBT inhibition with SC-435 restored glucose tolerance, reduced hepatic triglyceride and total cholesterol concentrations, and improved NAFLD activity score. These changes were associated with reduced hepatic expression of lipid synthesis genes and normalized expression of the central lipogenic transcription factor, Srebp1c [10].

Human studies with ASBT inhibition in NASH are lacking. A search on ClinicalTrials.gov revealed a study with Volixibat that still is recruiting patients.

Considering NASH as complication of the metabolic syndrome, in particular in those with type 2 diabetes mellitus (T2DM), the effect of ASBT inhibition in NASH probably needs to be compared with the effect of bile acid sequestrants that have similar effects on the bile pool and have been to improve metabolic control in a number of trials in T2DM [11].

References

[1] Dawson PA, Haywood J, Craddock AL, Wilson M, Tietjen M, Kluckman K, Maeda N, Parks JS. Targeted deletion of the ileal bile acid transporter eliminates enterohepatic cycling of bile acids in mice. J Biol Chem. 2003;278(36):33920-7.
[2] Arab JP, Karpen SJ, Dawson PA, Arrese M, Trauner M. Bile acids and nonalcoholic fatty liver disease: Molecular insights and therapeutic perspectives. Hepatology. 2017;65(1):350-62.
[3] Miethke AG, Zhang W, Simmons J, Taylor AE, Shi T, Shanmukhappa SK, Karns R, White S, Jegga AG, Lages CS, Nkinin S, Keller BT, Setchell KD. Pharmacological inhibition of apical sodium-dependent bile acid transporter changes bile composition and blocks progression of sclerosing cholangitis in multidrug resistance 2 knockout mice. Hepatology. 2016; 63(2):512-23.
[4] Baghdasaryan A, Fuchs CD, Osterreicher CH, Lemberger UJ, Halilbasic E, Pahlman I, Graffner H, Krones E, Fickert P, Wahlstrom A, Stahlman M, Paumgartner G, Marschall HU, Trauner M. Inhibition of intestinal bile acid absorption improves cholestatic liver and bile duct injury in a mouse model of sclerosing cholangitis. J Hepatol. 2016; 64(3):674-81.
[5] Graffner H, Gillberg PG, Rikner L, Marschall HU. The ileal bile acid transporter inhibitor A4250 decreases serum bile acids by interrupting the enterohepatic circulation. Aliment Pharmacol Ther. 2016;43(2):303-10.
[6] Mayo MJ, Pockros PJ, Jones D, Bowlus C, Levy C, Patanwala I, Bacon B, Luketic V, Vuppalanchi R, Medendorp S, Dorenbaum A, Kennedy C, Novak P, Raychaudhuri A, Goyal S, Abi-Saab W, Hirschfield GM. Clarity: A Phase 2, Randomized, Double-Blind, Placebo-Controlled Study of Lopixibat Chloride (Formerly Lum001), a Novel Apical Sodium-Dependent Bile Acid Transporter Inhibitor, in the Treatment of Primary Biliary Cirrhosis Associated with Itching. J Hepatol.64(Suppl 1):S197.
[7] Hegade VS, Kendrick SF, Dobbins RL, Miller SR, Thompson D, Richards D, Storey J, Dukes GE, Corrigan M, Oude Elferink RP, Beuers U, Hirschfield GM, Jones DE. Effect of ileal bile acid transporter inhibitor GSK2330672 on pruritus in primary biliary cholangitis: a double-blind, randomised, placebo-controlled, crossover, phase 2a study. Lancet. 2017;389(10074):1114-23.
[8] Al-Dury S, Wahlström A, Wahlin S, Langedijk J, Oude Elferink R, Ståhlman M, Marschall HU. Pilot study with IBAT inhibitor A4250 for the treatment of cholestatic pruritus in primary biliary cholangitis. Hepatology. 2017;Supplement
[9] Sturm E, Baumann U, Lacaille F, E. G, Arnell H, Fischler B, Hørby Jørgensen M, Thompson RJ, Mattsson JP, Ekelund M, Erik Lindström E, Gillberg PG, Kristina Torfgård K, Soni PN. The ileal bile acid transport inhibitor A4250 reduced pruritus and serum bile acid levels in children with cholestatic liver disease and pruritus: final results from a multiple-dose, open-label, multinational study. Hepatology. 2017; Supplement.
[10] Rao A, Kosters A, Mells JE, Zhang W, Setchell KD, Amanso AM, Wynn GM, Xu T, Keller BT, Yin H, Banton S, Jones DP, Wu H, Dawson PA, Karpen SJ. Inhibition of ileal bile acid uptake protects against nonalcoholic fatty liver disease in high-fat diet-fed mice. Sci Transl Med. 2016;8(357):357ra122.
[11] Hansen M, Sonne DP, Mikkelsen KH, Gluud LL, Vilsboll T, Knop FK. Bile acid sequestrants for glycemic control in patients with type 2 diabetes: A systematic review with meta-analysis of randomized controlled trials. J Diabetes Complications. 2017; 31(5):918-27.

Disclosure of Interest: H.-U. Marschall:Grant/Research Support: Albireo, Intercept, Consultant / Advisor: Albireo, Intercept, Sponsored Lectures: National or International: Intercept

28

Academic overview: Gut microbiota

Bernd Schnabl[*1]

[1]*Medicine, University of California San Diego, La Jolla, United States*

Corresponding author's email: beschnabl@ucsd.edu

Non-alcoholic fatty liver disease (NAFLD) has a high prevalence worldwide. Although nutrition plays a central role for disease onset and progression, disease pathogenesis is multifactorial including genetic and environmental factors. In addition, the gut microbiota is involved in disease progression and its role is complex. It has been recognized for a long time that chronic liver disease is associated with changes in the enteric microbiota and the gut barrier. Alterations in the microbiota can occur as quantitative (bacterial overgrowth) and compositional dysbiotic changes. Intestinal bacterial overgrowth is a common feature in patients with liver cirrhosis, but it also occurs in early stages of patients with non-alcoholic fatty liver (NAFL). Qualitative changes of the human gut microbiome have now been characterized by deep sequencing, and several studies described the microbial taxonomy in patients with early and endstage liver disease. How does intestinal dysbiosis contribute to progression of NAFL to non-alcoholic steatohepatitis (NASH)? Mucosal barrier dysfunction in the intestine and microbial metabolites are important for disease progression. Effects of untargeted microbiome modifications using antibiotics, probiotics and prebiotics on liver disease have been successfully tested in preclinical models. Overall evidence for effects in patients with chronic liver disease is promising, but sufficiently powered long-term clinical trials are required to assess outcome. There is a clear need to identify better therapeutic targets in the gut microbiota that can be utilized for precision and personalized medicine approaches in the treatment of NAFLD.

References

[1] Schnabl B, Brenner DA. Interactions between the intestinal microbiome and liver diseases. Gastroenterology. 2014; 146(6):1513-24.

[2] Bluemel S, Williams B, Knight R, Schnabl B. Precision medicine in alcoholic and non-alcoholic fatty liver disease via modulating the gut microbiota. Am J Physiol Gastrointest Liver Physiol. 2016; 311(6):G1018-G1036.

Disclosure of Interest: B. Schnabl: Grant/Research Support: NGM Biopharmaceuticals, Consultant / Advisor: HOST Therabiomics

29

Microbiota and FXR signaling in NAFLD and obesity to induce or not to induce – that is the question

Annika Wahlström* [1]

[1]*Department of Molecular and Clinical Medicine, Sahlgrenska University Hospital/Gothenburg University, Gothenburg, Sweden*

Corresponding author's email: annika.wahlstrom@wlab.gu.se

The gut microbiota influences the development and progression of metabolic diseases, such as obesity and type 2 diabetes mellitus (T2DM), partly by metabolism of bile acids and modified signaling through the farnesoid X receptor (FXR). Non-alcoholic fatty liver disease is the hepatic manifestation of the metabolic syndrome and results from dysregulated lipid and glucose metabolism. NAFLD is a complex disease spanning from simple steatosis to non–alcoholic steatohepatitis (NASH), which is characterized by steatosis with ballooning and various degrees of fibrosis that may progress to cirrhosis, and ultimately liver cancer [1]. The crosstalk between the gut microbiota and bile acids and its impact on signaling through FXR is under extensive investigation and targeting this interaction might be a future treatment option for the development and progression of NAFLD.

Studies in mice have generated somewhat conflicting results regarding the role of FXR in metabolic diseases. *Fxr*-deficient mice on chow diet develop hyperglycemia and hypercholesterolemia [2]. In contrast, it appears that *Fxr*-deficient mice bred on a genetically obese background or fed a high-fat diet are protected against obesity and show improved glucose homeostasis compared with wild type control mice [3] [4] [5]. Furthermore, studies using intestinal and hepatic disruption of FXR indicate that intestinal and hepatic FXR may have opposing effects on metabolic diseases and steatosis. Hepatic expression of FXR protects against hepatic steatosis and elevated triglyceride and bile acid levels [6] while intestinal-specific inhibition of *Fxr* protects against liver steatosis and obesity in mice fed a high-fat diet [7] [8].
To study the influence of microbiota and bile acid interactions on host metabolism we use germ-free mice that can be colonized with specific communities of bacteria. These mice are important tools but the interpretation and translation of results from mouse models must be done carefully since mice and humans have substantial differences in bile acid composition. The major primary bile acid in germ-free mice, TβMCA, is absent in adult humans and this bile acid function as an FXR antagonist [9]. It has been shown that mice treated with antibiotics or Tempol have increased levels of TβMCA and are protected against diet- induced obesity and show improvement in NAFLD and it was suggested that intestinal-specific inhibition of FXR was responsible for the beneficial effects [8]. A glycine-conjugated form of βMCA (GβMCA) has also been described, which improved the metabolic phenotypes in high-fat or genetically induced obesity mouse models [10] [11].

On the other hand, it has also been shown that intestinal-specific activation of FXR with fexaramine protects against the development of obesity and was associated with increased thermogenesis and browning of adipose tissue [12]. Taken together, it is not clear whether it is more beneficial to activate or to inhibit FXR and future studies are needed to clarify this especially in humans.

References

[1] Rinella, M.E., *Nonalcoholic fatty liver disease: a systematic review.* JAMA, 2015. **313**(22): p. 2263-73.
[2] Lambert, G., et al., *The farnesoid X-receptor is an essential regulator of cholesterol homeostasis.* J Biol Chem, 2003. **278**(4): p. 2563-70.

[3] Zhang, Y., et al., *Identification of novel pathways that control farnesoid X receptor-mediated hypocholesterolemia.* J Biol Chem, 2010. **285**(5): p. 3035-43.
[4] Prawitt, J., et al., *Farnesoid X receptor deficiency improves glucose homeostasis in mouse models of obesity.* Diabetes, 2011. **60**(7): p. 1861-71.
[5] Parseus, A., et al., *Microbiota-induced obesity requires farnesoid X receptor.* Gut, 2017. **66**(3): p. 429-437.
[6] Schmitt, J., et al., *Protective effects of farnesoid X receptor (FXR) on hepatic lipid accumulation are mediated by hepatic FXR and independent of intestinal FGF15 signal.* Liver Int, 2015. **35**(4): p. 1133-1144.
[7] Li, F., et al., *Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity.* Nat Commun, 2013. **4**: p. 2384.
[8] Jiang, C., et al., *Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease.* J Clin Invest, 2015. **125**(1): p. 386-402.
[9] Sayin, S.I., et al., *Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist.* Cell Metab, 2013. **17**(2): p. 225-35.
[10] Gonzalez, F.J., C. Jiang, and A.D. Patterson, *An Intestinal Microbiota-Farnesoid X Receptor Axis Modulates Metabolic Disease.* Gastroenterology, 2016. **151**(5): p. 845-859.
[11] Zhang, L., et al., *Farnesoid X Receptor Signaling Shapes the Gut Microbiota and Controls Hepatic Lipid Metabolism.* mSystems, 2016. **1**(5).
[12] Fang, S., et al., *Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance.* Nat Med, 2015. **21**(2): p. 159-65.

Disclosure of Interest: None Declared

30

Probiotic intervention in NAFLD

Vanessa Stadlbauer-Köllner* [1]

[1]*Department of Gastroenterology and Hepatology, Medical University of Graz, Graz, Austria*

Corresponding author's email: vanessa.stadlbauer@medunigraz.at

The gut microbiome is thought to play an important role in the pathogenesis of NAFLD and NASH. Therefore interventions to target the gut microbiome composition and function are attractive research topics. Probiotics are defined as 'live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host' by the WHO. Most probiotics have been isolated from human gut or are used in food processing. Generally, probiotics are considered to be safe. Safety assessment of probiotics is performed by the European Food Safety Authority. Potential risks are gastrointestinal discomfort, infections and metabolic side effects.
In NASH/NAFLD potential targets for probiotic intervention are dysbiosis, free fatty acid metabolism, permeability, intestinal inflammation, bacterial translocation and energy homeostasis. Targeting the gut-liver axis, especially metabolic endotoxemia and increased gut permeability seems to be promising.
The available data on probiotic therapy in NAFLD/NASH are discussed, starting from the first animal studies in 2003 that showed a reduction in aminotransferases and a decrease in liver fat content.
The available human studies are discussed: Most studies showed beneficial effects of various probiotic preparations on liver enzymes and surrogate parameters of glucose metabolism, lipid metabolism and oxidative stress. Only very recently the first studies with "hard" endpoints such as liver histology or at least non-invasive fibrosis or fat content assessments were presented, showing a positive effect in small pilot trials. The question whether combination of probiotics with other treatment strategies is useful, cannot be answered to date.
In conclusion targeting the gut-liver axis via probiotics in NAFLD/NASH seems to be a promising and safe strategy. Several unsolved research questions remain to understand the underlying mechanisms.

Disclosure of Interest: V. Stadlbauer-Köllner: Grant/Research Support: Fresenius Austria, Winclove GmbH, Institut Allergosan, Yakut Austria, Yakult Europe, Sponsored Lectures: National or International: Institut Allergosan, Yakult Austria, Fresenius Austria

32

miRNAs as biomarkers and targets

Pedro M. Rodrigues[1], Marta B. Afonso[1], Andre L. Simão[1], Rui E. Castro[1], Cecilia M. Rodrigues[*1]

[1]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

Corresponding author's email: cmprodrigues@ff.ul.pt

The transition from benign steatosis to NASH and hepatocellular carcinoma (HCC) occurs through yet unclear molecular mechanims. We have recently profiled miRNAs in murine liver samples and identified several changes with disease. In a diet model of NASH-associated liver damage, miR-21 ablation resulted in decreased steatosis, inflammation and lipoapoptosis, and prevented fibrosis. In a different diet model featuring the metabolic syndrome, miR-21 levels increased and its PPARα target decreased both in liver and muscle tissues. miR-21 ablation and FXR activation resulted in minimal hepatic steatosis, inflammation, oxidative stress and cholesterol accumulation. Lipoprotein metabolism was restored, and liver and muscle insulin sensitivity fully reinstated. The miR-21/PPARα axis was also amplified in liver and muscle biopsies, and in serum of NAFLD patients. Interestingly, we have also shown that liver damage and RIPK3-dependent necroptosis were prevented in bile duct-ligated *miR-21*$^{-/-}$ mice, via relieved repression of CDK2AP1. In a recent study, wild-type and *miR-21*$^{-/-}$ mice were fed either choline-sufficient, amino acid-defined control diet or choline-deficient, amino acid-defined diet (CDAA) for 32 and 66 weeks. Wild-type mice fed the CDAA diet for 32 weeks developed all main histological features of NASH. After 66 weeks, all mice on CDAA developed preneoplastic nodules and one animal trabecular HCC. Hepatocytes were highly proliferative and expressed high levels of pro-inflammatory/fibrogenic markers, particularlly in pre-neoplastic liver tissue. miR-21 expression was increased in CDAA-fed mice and further increased in HCC, while PPARα and its direct transcriptional genes were decreased. Strikingly, *miR-21*$^{-/-}$ mice fed the CDAA diet for 32 weeks showed decreased triglycerides and fatty acids in serum; after 66 weeks, serum transaminases were similar to controls, liver nodules decreased and the pro-inflammatory/fibrogenic milleau was reduced. Interestingly, RIPK3 ablation in itself halted long-term inflammation, fibrosis, hepatocyte proliferation, genetic resistance of dysplastic hepatocytes to cell death, oxidative stress and tissue microenvironment changes associated with NASH-driven hepatocarcinogenesis. By unveiling that miR-21 ablation significantly improves cell function and whole body metabolism, our results highlight the therapeutic potential of targeting multiple components of NAFLD pathogenesis.
(Funding: PTDC/BIM-MEC/0895/2014; SAICTPAC/0019/2015)

Disclosure of Interest: None Declared

33

Involvement of the immune system in NASH and NASH-to-HCC transition

Mathias Heikenwälder* [1]

[1]*Division of Chronic Inflammation and Cancer, German Cancer Research Center (DKFZ), Neuenheimer Feld, Heidelberg, Germany*

Corresponding author's email: heikenwaelder@helmholtz-muenchen.de

Due to consumption of high caloric food combined with increased sedentary lifestyle, overweight and obesity incidence has grown rapidly in Western cultures (e.g. USA, Europe) but notably also in developing countries (e.g. India, China). Although chronic viral infections with Hepatitis B or C are still the leading cause for hepatocellular carcinoma (HCC), alcoholic steatohepatitis (ASH), non-alcoholic fatty liver (NAFL) and subsequent non-alcoholic steatohepatitis (NASH) have become important etiologies for HCC. NASH and ASH greatly contribute to the fact that HCC is the fastest rising cancer in the USA, with a similar trend in Europe. We and others have generated several pre-clinical mouse models that enable studying the mechanisms of NASH development and NASH to HCC transition in the context of a metabolic syndrome. Remarkably, these models recapitulated several human pathophysiological hallmarks of NASH and HCC. It has become apparent that adaptive immune cells play an important role in driving NASH and HCC - but at the same time actively participate in tumor surveillance. Here, I will report on the characterization and identification of novel targets within a NASH model recently characterized in our laboratory.

Disclosure of Interest: None Declared

35

Liver histology for biomarker and target discovery

Peter Schirmacher[*1]

[1]*Institute of Pathology, University Hospital Heidelberg, Germany*

Corresponding author's email: peter.schirmacher@med.uni-heidelberg.de

Liver cancer (hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC)) mainly develops on the basis of chronic liver disease, mostly on established cirrhosis. Non-alcoholic fatty liver disease is emerging as one of main etiologies for liver cirrhosis and liver cancer, especially in Western industrialized countries. While precision medicine approaches have revolutionized modern cancer medicine and have resulted in many novel targets, therapeutic approaches, and diagnostic assays in different tumor diseases, in liver cancer they have not lead to approved therapies so far; the various reasons include improper trial design, inadequate drugs, and lack of trial-associated biopsy-driven biomarker approaches.Liver biopsy should be a mandatory prerequisite in all clinical trials in liver cancer, in accordance with current guidelines. It allows for histology-based diagnostic validation of the respective tumor as well as tumor tissue based analyses of potentially predictive biomarkers. Meanwhile all relevant single marker (IHC, FISH) and multimarker approaches (expression profiling, NGS, methylome analysis) have been successfully adapted to FFPE tissue, biopsy tissue size and routine diagnostic work flows and therefore are amenable to all different clinical trial scenarios, such as upfront (stratifying) diagnostics as well as trial associated analyses/research.Appropriate test-selection in preclinical research and clinical trials is critical for the success of any targeted drug as it defines later real world testing scenario; it has to assure sufficient sensitivity and specificity, robustness, short TATs, financial feasibility, and broad applicability of the test in order to reach broad coverage and acceptance by the diagnostic community together with realistic cost scenarios, all of which is mandatory for identifying those patients amenable to the specific treatment. In contrast to general recognition, the transfer of an experimentally identified and trial-proven drug target into the real world diagnostic scenario is a tremendous effort with many hurdles that are frequently insufficiently reflected and addressed until late clinical trials.Both main types of liver cancer, HCC and ICC, in principle offer sufficient number and frequencies of molecular therapeutic targets. Targeted drug approaches combined with predictive testing have been carried out for hepatocellular carcinoma (e.g. MET, KRAS) but have not been successful so far; several further approaches (e.g. addressing FGFR, PRKACA, MET) are ongoing. ICC offers many attractive therapeutic targets at overall high frequency (e.g. FGFR2-TLs, IDH-, NTRK-, BRAF-mutations, MSI) which are aimed at by ongoing clinical trials. Both cancer types are the target of tumor immunology oriented trials using checkpoint inhibitors; MSI-cancer is infrequent but detectable in ICC, and further trial associated research is necessary to establish a potential role of specific predictive testing for immunotherapy in liver cancer (e.g. PD-L1, MSI, mutational load etc.).It should be emphasized that biopsy-based biomarker discovery and testing should not be limited to targeted therapeutic approaches; positive and negative predictors are equally relevant for non-targeted approaches, such as chemo- and virotherapy. Finally, with the advent of second and later line treatment approaches, testing for molecular resistance mechanisms – as already established in NSCLC – may also become a reality.Center-based approaches to improve the dismal situation of precision medicine in liver cancer involving broad tumor testing strategies (so-called 'umbrella concepts') have been implemented in order to improve patient allocation to clinical trials, trial planning strategies, experimental treatment concepts (molecular tumor boards) and to foster translational research. These concepts require active biopsy and recall policies, patient and trial registries, comprehensive molecular testing by strong molecular pathology units and interdisciplinary trial/tumor boards integrating all findings. Active biopsy strategies further support biobanking activities and bedside-bench translational research.

Disclosure of Interest: None Declared

36

Academic overview: Liver fibrosis

Christian Trautwein* [1]

[1]*Medizinische Klinik III, RWTH Aachen, Germany*

Corresponding author's email: ctrautwein@ukaachen.de

Chronic liver injury can be triggered different etiologies, e.g. non-alcohlic liver disease (NAFLD), which induces repetitive tissue damage. Consequently the liver cannot simply replace its organ mass and a uniform etiology-independent process is activated. This process is characterized by the infiltration and activation of immune cells, leading to both inflammatory as well as wound healing responses. This includes replacement by connective tissue and extracellular matrix material (ECM). Although initially beneficial, the wound healing process becomes pathogenic if it progressively replaces parenchyma with scar tissue. The excessive deposition of ECM and loss of parenchymal cells lead to progressive destruction of organ architecture, eventually resulting in organ dysfunction. Liver fibrosis in each patient is not a uniform process, but is influenced by environmental and genetic factors. Recent work has identified specific genes as essential and mostly etiology-independent risk factors of fibrosis progression. Genetic factors contribute to around 50% of fibrosis progression and thus play a relevant risk of each patient to have slower or faster fibrosis progression. Some of these factors have been first described in cohorts of patients with NAFLD. Important genetic factors are variations in the genes of PNPLA3, interferon-λ4, TM6SF2, MBOAT7 and MERTK. Interestingly some of these genes are involved in fat metabolism and therefore lead to higher cellular stress and death of hepatocytes enhancing liver injury.Under physiological conditions, the liver is an essential organ in mediating tolerance. Here local macrophages, Kupffer cells, play a central role for liver immune homeostasis.

They are important to control and dampen mechanism activating the inflammatory response. However when insults e.g. during metabolic stress or hepatocyte injury trigger the intracellular thresholds of tolerance they change their activating status and play a central role in regulating the local inflammatory response. They secrete mediators to coordinate the stimulation status of other cell types, e.g. HSCs, NKT cells and infiltrating monocytes/macrophages. These mediators include chemokines, cytokines and growth factors. Through these mechanism they also coordinate the influx of immature monocyte-derived $Ly6C^{hi}$ macrophages. Pro-inflammatory $Ly6C^{hi}$ macrophages are of major relevance for fibrosis progression and thus fuel this process as their deletion e.g. in Cd11b-DTR transgenic mice, inhibits the profibrogenic response. $Ly6C^{hi}$ macrophages can differentiate into pro-resolution/restorative $Ly6C^{lo}$ macrophages. Pathways mediating this switch, e.g. the fractalkine receptor CX_3CR1 are interesting as they are involved in changing from progression to resolution of liver fibrosis. Fibrolytic $Ly6C^{lo}$ macrophage secrete metalloproteinases such as MMP-9 and MMP-13 as well as the anti-fibrotic cytokine IL-10, thereby significantly contributing to fibrosis resolution.

Early clinical trials using macrophage subset cell transfer aim at translating these results into novel treatment for human disease.Recent advances in the field have demonstrated that the hepatic stellate cell (HSC) by its transformation into myofibroblasts (MFB) contributes to more than 90% of ECM production. HSC are located in the space of Disse adjacent to hepatocytes and endothelial cells. In their quiescent state they store Vitamin A in fat droplets. Upon chronic injury they lose their lipid droplets and proliferate. In their activated form HSCs as MFB are believed to have a minor role in maintaining the inflammatory response during chronic liver injury, even they are able to release several inflammatory mediators. The activation of intracellular pathways in MFBs seems mainly important to control the production of ECM proteins and to secure survival of HSCs via e.g. NF-kB- and AP-1.

The concept of fibrosis reversibility is striking and closely linked to findings using fate tracing experiments to monitor the plasticity of HSCs. HSCs after MFB transdifferentiation are able to reverse their phenotype back to a phenotype similar to quiescent HSCs. During fibrosis resolution only a certain yet not defined percentage of MFB reverse into HSCs, while the remaining MFBs are triggered to become apoptotic. Most likely the lack of pro-survival signals in a non-fibrogenic liver environment is responsible for this observation. Therefore, future approaches will aim to trigger reversal of MFBs in order to treat patients with fibrosis.In summary chronic liver injury by e.g. NAFLD triggers a uniform process leading liver scarring. Here several cell types play distinct roles and the increasing knowledge of their functional roles leads to new treatment options in the clinic.

Disclosure of Interest: None Declared

37

Fibrosis as prognostically relevant signature: do we have to care about differentiating NASH from NAFL (or just fibrosis)

Mattias Ekstedt*[1]

[1]Linköping University, Linköping, Sweden

Corresponding author's email: mattias.ekstedt@liu.se

In my talk I will focus on histopathological predictors of mortality, liver-related complications and fibrosis progression. Studies during the last decade have established NAFLD as a potentially progressive liver disease. However, the natural history of NAFLD is still somewhat unclear. Older studies reported limited numbers of highly selected patients, i.e. patients with histologically proven NAFLD who had been referred to specialized tertiary care centers with short and/or highly variable follow-up time. There was, and still is, a shortage of population-based studies to determine the long-term prognosis of NAFLD and, it remains uncertain whether previously reported morbidity rates could be generalized to community-based practice where patients may have a milder disease.
Predicting fibrosis progression using baseline biochemical, clinical or histological parameters is difficult. Presence of NASH or high NAS at baseline has in previous studies not been associated with fibrosis progression, although NASH or high NAS were more prevalent in the follow-up biopsies in patients with disease progression.
Age and diabetes are the strongest clinical predictors of progressive disease. Fibrosis stage is the most important histological variable to predict mortality and liver-related complications.
There is convincing evidence that hepatic inflammation, cell death, lipotoxicity and insulin resistance is drivers of fibrogenesis in NAFLD. The lack of association between baseline NASH and progressive fibrosis is therefore puzzling.
The definition of NASH has varied over the years making it nearly impossible to compare studies from different time points. Moreover, the hallmarks of steatohepatitis, i.e. hepatocyte ballooning and lobular inflammation has considerable inter- and intra-variability. So far, no definition of NASH has been derived from its ability to predict progressive disease.
I will argue that before we make clinical decisions based on the presence of steatohepatitis we need data that has validated that we can predict clinical outcomes.

Disclosure of Interest: None Declared

38

How to non-invasively assess fibrosis (serum, transient elastography, MRE)?

Laurent Castera[*1]

[1]*Service d'Hépatologie, Hôpital Beaujon, Clichy, France*

Corresponding author's email: laurent.d.castera@gmail.com

Liver biopsy, the traditional "gold" standard for the staging of fibrosis in chronic liver disease, has been challenged over the past decade by non-invasive methods. These methods rely on two distinct but complementary approaches: a "biological" approach, based on the quantification of biomarkers of fibrosis in serum, and a "physical" approach, based on the measurement of liver stiffness using either elastography-based technologies (for which transient elastograpy (TE) (FibroScan) (Echosens, Paris, France) has been the pioneer) or magnetic-resonance elastography (MRE). Advantages of serum biomarkers include their high applicability (>95%) and good reproducibility. However, as none are liver specific their results can be influenced by co-morbid conditions and ALT levels. TE has the advantage of being a user's friendly procedure that can be performed at the bedside or in an outpatient clinic with high performance for detecting cirrhosis. However, its applicability is lower (80%) than that of serum biomarkers (particularly in case of ascites, obesity and limited operator experience) with the risk of false positive results in case of ALT flares. Nevertheless, TE is currently the most widely available and validated technique worldwide, better at ruling out than ruling cirrhosis. MRE is probably the most accurate non-invasive technique for staging fibrosis but, given its high cost and limited availability, it remains a tool for research or clinical trials. Given the high prevalence of NAFLD worldwide, non-invasive methods are becoming increasingly used as triage of NAFLD patients who may require a liver biopsy.

Disclosure of Interest: L. Castera: Consultant / Advisor: Gilead, MSD, Sirtex, Promethera, Sponsored Lectures: National or International: Echosens, Sirtex

39

Targeting liver fibrosis: Have we been listening to this song for too long now? - Industry perspective

Matthias Ocker* 1

[1]*Oncology Biomarker Strategy, Bayer AG, Berlin, Germany*

Corresponding author's email: matthias.ocker@bayer.com

Chronic liver diseases leading to fibrosis have become a major epidemiologic and medical burden globally. As fibrotic liver disease could lead to severe organ impariment and also liver cancer development, developing early detection and surveillance strategies for diagnosis are urgently needed. Esp NAFLD is a complex syndrome with various underlying etiologic factors like metabolic changes but does also include inflammatory and immunologic components. Thus, development of disease specific biomarkers need to be developed in parallel to novel targeted tratment options. Here, target discovery and understanding of the etologic factors need to be improved. Successful translational and clinical development of such targeted drug candidates are currently also limited by the lack of specific companion biomarker programs and an uncertainty about regulatory endpoints for clinical trials and drug approval. This is closely linked to long trial durations due to potentially slow course of the disease and emphasizes that identification of the right patient population is crucial for the success of drug development in this area.
Academia and industry as well as consortia and regulatory agenies must therefore continue and improve their collaboration to overcome hurdles that hampered fibrosis drug development in the past.

Disclosure of Interest: M. Ocker: Stockholder: Bayer AG, Employee: Bayer AG

ORAL ABSTRACT PRESENTATIONS

O1

Omega-3 PUFA modulate lipogenesis, ER stress, and mitochondrial dysfunction markers in NASH – Proteomic and lipidomic insight

Lívia S. R. R. Okada[1], Claudia P. Oliveira[*2], José Tadeu Stefano[2], Monize A. Nogueira[2], Ismael D. C. G. Silva[3], Fernanda B. Cordeiro[4], Venâncio A. F. Alves[5], Raquel S. Torrinhas[1], Flair José Carrilho[2], Puneet Puri [6], Dan L. Waitzberg[1]

[1]gastroenterology (LIM-35), [2]gastroenterology (LIM-07), University of Sao Paulo school of medicine, [3]Gynecology, [4]Urology, Sao Paulo Federal University, [5P]athology (LIM-14), University of Sao Paulo school of medicine, Sao Paulo, Brazil, [6]Virginia Commonwealth University-VCU, Virginia, United States

Corresponding author's email: claudia.oliveira220@fm.usp.br

Introduction: Currently there is no FDA-approved therapy for nonalcoholic steatohepatitis (NASH). Increased n-6/n-3 polyunsaturated fatty acids (PUFA) ratio can induce endoplasmic reticulum (ER) stress and mitochondrial dysfunction that characterize NASH. Our recent study with n-3 PUFA showed improvement in individual histologic parameters like steatosis, ballooning and lobular inflammation.

Aims: We hypothesized that n-3 PUFA therapy mediated improvement in histologic parameters is modulated by lipidomic and proteomic changes.

Material and Methods: We therefore evaluated hepatic proteomic and plasma lipidomic profiles before and after n-3 PUFA therapy in subjects with NASH. In a double-blind, randomized, placebo-controlled trial, patients with NASH received 6-month treatment with n-3 PUFA (0.945 g/day [64% alpha-linolenic (ALA), 21% eicosapentaenoic (EPA), and 16% docosahexaenoic (DHA) acids]). Paired liver biopsy and plasma collected before and after-n-3 PUFA therapy were assessed using mass spectrometry and gas chromatography for hepatic proteomics and plasma lipidomics. Data were matched to UniProt and LIPID MAPS database, respectively. Cytoscape software was used to analyze functional pathways. Twenty-seven NASH patients with paired liver histology and plasma before and after n-3 PUFA treatment were studied.

Results: Treatment with n-3 PUFA significantly increased ALA, EPA, and glycerophospholipids, and decreased arachidonic acid ($p < 0.05$ for all). Further, proteomic markers of cell matrix, lipid metabolism, ER stress and cellular respiratory pathways were also modulated. Interestingly, these alterations reflected functional changes highly suggestive of decreased cellular lipotoxicity potential; reduced ER proteasome degradation of proteins and induction of chaperones; and a shift in cell energy homeostasis towards mitochondrial beta-oxidation.

Conclusions: ix-month treatment with omega-3 PUFAs significantly improved hepatic proteomic and plasma lipidomic markers of lipogenesis, endoplasmic reticulum stress and mitochondrial functions in patients with NASH.

Disclosure of Interest: None Declared

O2

Impact of alive probiotics supplementation with absorbent smectite gel in NAFLD management: evidence from animals to randomized clinical studies

Nazarii Kobyliak[*] [1], Tetyana Falalyeyeva[2], Galyna Mykhalchyshyn[1], Dmytro Kyriienko[3], Oleg Dynnyk[4], Iuliia Komissarenko[1]

[1]Bogomolets National Medical University, [2]Taras Shevchenko National University of Kyiv, [3]Kyiv City Clinical Endocrinology Center, [4]Bogomolets Institute of physiology of the Ukrainian National Academy of Sciences, Kyiv, Ukraine

Corresponding author's email: nazariikobyliak@gmail.com

Introduction: We have previously shown that in rats with momosodiume glutamate induced obesity supplementation of alive probiotics with smectite gel due to his absorbent activity lead to lower total NAS (NAFLD activity score) score, with more pronounced reduction of lobular inflammation (0.13±0.09 vs 0.33±0.15) as compared to administration of probiotic alone.

Aims: In respect to our experimental data we studied, in double-blind single center randomized clinical trial (RCT), the efficacy of alive probiotics supplementation with smectite gel vs. placebo in type 2 diabetes patient with NAFLD detected on ultrasonography (US).

Material and Methods: A total of 50 patients met the criteria for inclusion. They were randomly assigned to receive Symbiter Forte" combination of probiotic biomass with smectite gel (250 mg) or placebo for 8-weeks administered as a sachet formulation in double-blind treatment. The primary main outcomes were the change in fatty liver index (FLI) and liver stiffness (LS) measured by Shear Wave Elastography (SWE). FLI a validated prediction score for hepatic steatosis severity designed Bedogni et al. Secondary outcomes were the changes in transaminases activity, serum lipids and cytokines (TNF-α, IL-1β, IL-6, IL-8, INF-γ) levels. ANCOVA was used to assess the difference between groups.

Results: All subjects completed the study and received more than 90% of prescribed sachets. In respect to our primary endpoints, FLI and LS measured by SWE insignificant decrease in both interventional and placebo groups. However, when we compare mean changes across groups from baseline, expressed in absolute values, the reduction of both FLI (-0.750±1.23 vs 3.769 ±1.84; p=0.048) and LS (-0.254±0.85 vs 0.262 ±0.77; p=0.031) were observed. Analysis of secondary outcomes showed that co-administration of probiotic with smectite lead to significant reduction of ALT, total cholesterol (TC), IL-1β (39.28±3.59 vs 35.66 ±3.13; p=0.029) and TNF-α (49.66±3.51 vs 42.31±3.05; p<0.001) as compared to week 8. In placebo group changes were insignificant.

Conclusions: In this RCT, we confirmed previously reported by us animal data, that co-administration of probiotic with smectite due to his absorbent activity and stabilization mucus layer properties can impact on synergistic enhancement of single effect which manifested with reduction of liver fat and chronic systemic inflammation, and metabolic profile improvement.

Disclosure of Interest: None Declared

ePOSTER ABSTRACT PRESENTATIONS

P01-01

Soluble basigin levels increase with liver disease progression from NAFLD to NASH

Aimei Lee[*1, 2, 3, 4, 5], Susan McLennan[6, 7], Nicholas Shackel[3, 4, 5]

[1]Liver Cell Biology, Centenary Institute of Cancer Medicine and Cell Biology, [2]Sydney Medical School, The University of Sydney, Camperdown, [3]Gastroenterology and Liver Laboratory, Ingham Institute for Applied Medical Research, Liverpool, [4]Faculty of Medicine, University of New South Wales, Kensington, [5]Department of Gastroenterology and Hepatology, Liverpool Hospital, Liverpool, [6]Department of Endocrinology, Royal Prince Alfred Hospital, [7]Endocrinology, The University of Sydney, Camperdown, Australia

Corresponding author's email: aimei.lee@unsw.edu.au

Introduction: Basigin is a glycoprotein involved in extracellular matrix remodelling and has a role in the recruitment of inflammatory cells to sites of injury. Membrane-bound Basigin on cells has been shown to be increased in many diseases, including liver fibrosis, cirrhosis and liver cancer.

Aims: The aim of this study was to measure soluble Basigin levels in plasma from patients with different liver diseases to elucidate its expression in disease progression.

Material and Methods: This study recruited 130 patients; non-liver disease (n = 19), non-alcoholic fatty liver disease (NAFLD) (n = 90), and non-alcoholic steatohepatitis (NASH) (n = 21). Clinical variables were measured including liver function enzymes, body mass index (BMI), and cholesterol levels. Soluble Basigin (ng/mL) was measured in plasma collected from these patients using an immunoassay.

Results: There was a significant increase in soluble Basigin in the NASH group compared to the non-liver disease control group (median values 32.78ng/mL vs 40.45ng/mL, respectively; $p < 0.05$). There was also a significant increase in soluble Basigin between the patients in the NAFLD group compared to the NASH group (median values 31.71ng/mL vs 40.45ng/mL, respectively; $p < 0.05$). The BMI of the NAFLD and NASH groups was significantly higher than the non-liver disease control group (median values 36.55kg/m^2 and 41.90kg/m^2, respectively vs 23.30 kg/m^2). Soluble Basigin levels also correlated positively to BMI in the NAFLD group ($r = 0.50$, $p < 0.0001$).

Conclusions: Basigin levels in the circulation increases with liver disease. Basigin levels also increase with worsening disease as demonstrated by the higher levels of Basigin detected in NASH patients compared to NAFLD patients. Utilising Basigin detection in the blood may indicate changes to the patient's disease state.

Disclosure of Interest: None Declared

P01-02YI

The polygenic determinants of non-alcoholic fatty liver disease (NAFLD): A comprehensive evaluation by a re-sequencing approach

Alessia Di Costanzo[*1], Francesca Belardinilli[2], Diego Bailetti[3], Marialuisa Sponziello[4], Laura D'Erasmo[4], Francesco Baratta[4,5], Licia Polimeni[4], Fabrizio Ceci[6], Gabriella Girelli[7], Bruna De Masi[7], Antonio Angeloni[2], Giuseppe Giannini[2], Maria Del Ben[4], Marcello Arca[4], Francesco Angelico[8]

[1]Internal Medicine and Medical Specialties, Sapienza University of Rome, [2]Molecular Medicine, [3]Experimental Medicine, [4]Internal Medicine and Medical Specialties, [5]Anatomical, Histological, Forensic Medicine and Ortopedics, [6]Cellular Biotechnologies and Hematology , [7]Immunohematology and Transfusion Medicine Unit, [8]Public Health and Infectious Disease, La Sapienza University, Rome, Italy

Corresponding author's email: alessia.dicostanzo@uniroma1.it

Introduction: NAFLD is a polygenic condition but the individual as well as the cumulative contribution of identified gene variants remain to be established.

Aims: Here we performed a targeted sequencing of *GCKR, PPP1R3B, NCAN, LYPLAL1* and *TM6SF2* genes to comprehensively characterize the genetic architecture of NAFLD.

Material and Methods: Next generation sequencing was used to screen 218 subjects with ultrasound-defined NAFLD and 227 controls previously genotyped for PNPLA3 variants.

Results: A total of 168 sequence variants were detected; 68 were exonic and 47 were annotated as functional based on *in silico* prediction. When the cumulative frequencies of all functional variants within each gene were considered, only those in *TM6SF2* significantly accumulate in NAFLD subjects compared to controls (*P*=0.04). Among individual variants, rs1260326 C/T in *GCKR (recessive)*, rs58542926 C/T in *TM6SF2* and rs738409 C/G in *PNPLA3 (dominant*) emerged as significantly associated to NAFLD, with the *PNPLA3* being the best genetic predictor (OR 3.12, 95% CI 1.8-5.5; *P*<0.001). A 3-SNPs weighted genetic risk score value > 0.32 was associated with a five-fold increased risk of NAFLD. Interestingly rs738409 in *PNPLA3* gene associated with any degree of hepatic steatosis (all *P*≤0.05). Notably a NGS-identified variant in *PPP1R3B* gene (rs61756425 G/T p.S41R) was found to confer an increased susceptibility to severe NAFLD (OR, 26.4, 95%CI, 3.7-186.2, *P*=0.001).

Conclusions: We confirmed that *TM6SF2, GCKR* and *PNPLA3* are the best genetic predictors of NAFLD. Variants in the *PPP1R3B* might have a separate role in influencing the severity of liver fat.

Disclosure of Interest: None Declared

P01-04

Multifocal fatty infiltrative liver disease in obese subjects is associated with insulin resistance and enhanced systemic inflammation

Amr S. Hanafy[*1]

[1]*hepatogastroenterology, Zagazig University, zagazig, Egypt*

Corresponding author's email: Dr_amr_hanafy@yahoo.com

Introduction: Multifocal fatty liver with fatty sparing is a common ultrasonographic finding. The etiology of this phenomenon was explained by the presence of aberrant or normal veins that enter the liver directly independent of the portal venous system and communicate with intrahepatic portal branches to a variable degree as cholecystic veins, the epigastric-periumbilical veins and the peribiliary venous system of Couinaud, the latter originates from the pancreaticoduodenal and pyloroduodenal veins and runs along the common bile duct and the hepatic artery, it drains the head of the pancreas with the possibility of delivery of higher concentrations of insulin than that delivered by portal venous system leading to hepatic steatosis.

Aims: We aimed to investigate the clinical significance underlying the presence of this entity and if correlated with insulin resistance and enhanced systemic inflammation.

Material and Methods: An observational case control study included ***a study group*** composed of 96 patients with multifocal fatty liver. They were tested for Fasting blood glucose, triglycerides, ALT, AST, GGT, Serum insulin, HbA1c, and HOMA-IR, Mean platelet volume, neutrophil lymphocyte ratio (NLR), serum uric acid, ferritin, and RBP-4 levels. Liver stiffness measurement (LSM) by fibroscan was performed for the patients. These variables were reevaluated 6 months after therapy by vitamin E, pioglitazone, metformin. ***Two control groups***; one composed of 100 healthy subjects and the other group composed of 100 patients with diffuse fatty liver disease.

Results: *Multifocal fatty liver* (n=96) showed a statistically significant higher values of AST, ALT, GGT, FBS, Insulin, HbA1c, HOMA-IR, MPV, and NLR ($p<0.05$). RBP4, Ferritin, Uric acid and triglycerides were significantly higher ($p<0.05$). Serum AFP and mean LSM were significantly higher (7.5 ± 2.3ng/dl), (8.3 ± 2.2kpa) respectively when compared to healthy subjects and patients with diffuse fatty liver disease. After 6 months of therapy all the variables showed highly significant reduction with dramatic improvement in inflammatory, metabolic markers, and LSM value.

Conclusions: *Multifocal fatty liver* should be considered a radiological sign of insulin resistance and may indicate more advanced histological features within this type of steatosis which is highly sensitive to pioglitazone and metformin.

Disclosure of Interest: None Declared

P01-05YI

Role of mitofusin-2 in NAFLD and targeting by miRNAs

André L. Simão[*] [1], Marta B. Afonso[1], Pedro M. Rodrigues[1], Andreia J. Amaral[2], Margarida Gama-Carvalho[2], Antonio Zorzano[3], Cecília M. Rodrigues[1], Rui E. Castro[1]

[1]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, [2]BioFIG - Center for Functional and Integrative Genomics, Faculty of Sciences, Universidade de Lisboa, Lisbon, Portugal, [3]Institute for Research in Biomedicine (IRB Barcelona), Barcelona, Spain

Corresponding author's email: adlsimao@ff.ul.pt

Introduction: Non-alcoholic fatty liver disease (NAFLD) associates with intracellular lipid accumulation in the liver. Recent evidence supports a functional role for both miRNA and mitochondrial dysfunction in regulating NAFLD pathogenesis. In particular, deregulation of mitochondrial dynamics proteins, like mitofusin-2 (Mfn2), were recently described in obese and diabetic patients, while liver-specific Mfn2 silencing in mice promotes numerous metabolic abnormalities.

Aims: Our aims were to profile global liver miRNA expression changes during NAFLD and correlate them with the development of mitochondrial dysfunction in experimental and human NAFLD.

Material and Methods: C57BL6/N mice were fed either a standard or a fast food (FF) diet for 25 weeks, or a methionine and choline-deficient (MCD) diet for 2 and 8 weeks. Liver RNA from 8 weeks MCD-fed mice was run in TaqMan miRNA arrays. Liver biopsies were obtained from NAFLD patients with steatosis or nonalcoholic steatohepatitis (NASH). mRNA and protein expression were analysed by qRT-PCR and immunoblotting, respectively. miRNA targeting was evaluated by dual-luciferase reporter assays in HepG2 cells.

Results: FF-fed mice developed steatosis, inflammation and insulin resistance. MCD-fed mice developed progressive steatohepatitis and fibrosis. Liver Mfn2 mRNA and protein levels were significantly decreased in both MCD- and FF-fed animals, comparing with control diet-fed mice. Inversely, expression of dynamin-related protein-1 (Drp1), a mitochondrial fission protein, was found increased. In humans, liver Mfn2 protein levels decreased from steatosis to NASH. Twenty-five miRNAs were found significantly increased in the liver of MCD-fed mice. Inversely, 27 miRNAs were decreased. Among the increased miRNAs, 4 have at least one targeting Mfn2 3´UTR binding site in Mfn2 mRNA (miR-134, miR-125a, miR-222 and miR-34a). Dual-luciferase reporter assays confirmed targeting of Mfn2 by miR-125a, miR-222 and miR-34a in liver cells. Further, overexpression of either of these miRNAs in HepG2 cells inhibited Mfn2 expression.

Conclusions: Altogether, inhibition of Mfn2 constitutes a key mitochondrial dysfunction event during NAFLD triggering and progression. A better understanding of miRNAs modulated during NAFLD that directly target Mfn2 might aid in the elucidation of novel molecular pathways for therapeutic intervention. (SFRH/BD/104160/2014, FCT, PT and Gilead Sciences International Research Scholars Program 2015).

Disclosure of Interest: None Declared

P01-06

Controlled Attenuation Parameter (CAP) for point of care assessment of steatosis in real life clinical practice. A prospective evaluation with M probe

Andrea Salmi*[1], Pietro Campagnola[2], Clarissa Ferrari[3], Luca Frulloni[2]

[1]Liver Unit, San Camillo, Brescia, [2]UO Gastroenterologia B, Azienda Ospedale Università, Verona, [3]Statistic unit, IRCCS San Giovanni di Dio Fatebenefratelli, Brescia, Italy

Corresponding author's email: andrea.salmi@tin.it

Introduction: In clinical practice the use of imaging and liver biopsy is changing. Risk stratification and prognosis of liver disease is accepted for HCV, HBV etiology and proposed for NAFLD (non-alcoholic fatty liver disease) patients (pts) through non-invasive fibrosis and fatty liver assessment like liver stiffness measurement (LSM) by transient elastography (TE) who can be performed together with Controlled attenuation parameter (CAP); an evaluation of these non-invasive methods need to be addressed and optimal cut off defined.

Aims: We aimed to compare our real life experience results with recently proposed validity criteria for CAP median dB\m and CAP-IQR>40.

Material and Methods: 638 patients with disease Etiology: HCV n377 (61.5%), NAFLD n 132(21.5%), HBV n40 (6.5%) OTHERs n64 (10.4%) were examined by FibroScan ®CAP (Echosense Paris): Ultrasound based TE for non-invasive diagnosis of fibrosis (kPa), simultaneously with CAP (dB\m) for diagnosis of degree of steatosis. CAP value groups defined as recently proposed (1-2):S0 (<248) S1 (248), S2 (268), S3 (280) cut off and CAP IQR (<40 value) in a prospective cohort were analyzed by ANOVA test for continuous variable and Chi squared test for categories variable. The median number of valid determination were 23.7 instead of 10 usually suggested.

Results: Technical failure in 1 case for obesity grade3, 24 pts (3.8%) excluded for TE-IQR/TE-Med >30%. In Table:In 613 pts steatosis distribuition was 47.6%, 13.2%,7%,30.8% for S0,S1,S2,S3 ; CAP-median dB\m cut off >280 (S3) was associated with male gender , high TE-mediankPa value, in 25.4% of HCV and 57% of NAFLD ethiology. Mean TEkPa median value distribuition was 8,8;7,7;7,9;10,3 for S0,S1,S2,S3 (P=0.050). In 27% of 132 NAFLD pts CAP-IQR > 40 occurred with mean total valid measures of 26.4. TE-kPa median value >10.5(12%), 7.0-10.5(11%), <7 (77%) defining high, intermediate, low risk pts (see details in Table).

Conclusions: In this cohort applying CAP-IQR > 40, 1\4 of pts could have low validity steatosis grade stratification but global prevalence of S3 statosis was high in NAFLD and HCV etiology. TE-kPa median value cut off adopted show possible biopsy indication in 11% of NAFLD pts.
1) Karlas T. et al http://dx.doi.org/10.1016/j.jhep.2016.12.022.
2) Wong VW et al http://dx.doi.org/10.1016/j.jhep.2017.05.005

Figure:

All patiets N=613		Total	CUT OFF CAP-median dB/m				Test group comperison
			≤ 248 N=292	>248 ≤268 N=84	>268 ≤280 N=43	>280 N=194	
Gender	F n(%)	296 (48.3%)	170 (58.2%)	45 (53.6%)	14 (32.6%)	65 (35%)	χ2=38.5, p<0.001
	M n(%)	317 (51.7%)	122 (41.8%)	36 (46.4%)	29 (67.4%)	124 (65%)	
Age (years)	mean (SD)	60.8 (13.6)	60.9 (14.0)	58.4 (13.2)	59.1 (15.7)	62.1 (12.5)	F=1.8, p=0.150
Diagnosis NAFLD,	n (%)	132 (21.5%)	28 (9.6)	18 (21.4)	10 (23.3)	76 (39.2)	χ2=68.5, p<0.001
HCV,	n (%)	377 (61.5%)	206 (70.5)	50 (59.5)	25 (58.1)	96 (49.5)	
HBV,	n (%)	40 (6.5%)	17 (5.8)	10 (11.9)	3 (7.0)	10 (5.2)	
Other,	n (%)	64 (10.4%)	41 (14.0)	6 (7.1)	5 (11.6)	12 (6.2)	
Total measures	mean (SD)	28.4 (17.0)	27.6 (16.1)	30.9 (16.8)	25.8 (14.6)	29.2 (18.7)	F=1.3, p=0.265
Valid measures	mean (SD)	23.7 (13.2)	24.5 (13.4)	26.2 (14.3)	22.8 (12.6)	21.8 (12.3)	F=2.8, p=0.038
Exam duration (sec)	mean (SD)	166 (181)	162(203)	164 (147)	146 (108)	178 (171)	F=0.5, p=0.663
TE kPa,median	mean (SD) [IQR]	9.1 (8.5) [5.3]	8.8 (8.0)	7.7 (4.7)	7.9 (8.1)	10.3 (10.2)	F=2.6, p=0.050
TE kPa IQR	mean (SD)	1.6 (2.2)	1.5 (1.5)	1.3 (0.9)	1.4 (1.9)	2.1 (3.1)	F=4.7, p=0.003
TE kPa IQR /TE kPa median (%)	mean (SD)	17.2 (8.5)	16.8 (7.4)	17.0 (9.7)	16.8 (6.9)	18.0 (9.6)	F=0.9, p=0.465
Se escludo quelli >30 % che sono n=24)	mean (SD)	16.3 (6.5)	16.2 (6.5)	15.8 (6.1)	16.3 (6.2)	17.0 (6.8)	F=0.4, p=0.727
CAP median (dB/m)	mean (SD)	254 (61)	205 (38.8)	258 (5.8)	274 (3.4)	322 (31.5)	F=517, p<0.001
CAP IQR (dB/m)	mean (SD)	38.2 (18.5)	42.1 (20.9)	38.1 (14.3)	34.5 (13.6)	33.0 (15.6)	F=10.5, p<0.001
NAFLD patients N= 132		Total	CUT OFF CAP-median dB/m				Test group comperison
			≤ 248	>248 ≤268	>268 ≤280	>280	
Gender	F n(%)	46 (34.8%)	15 (53.6%)	9 (50.0%)	2 (20%)	20 (26.3%)	χ2=9.6, p=0.023
	M n(%)	86 (65.2%)	13 (46.4%)	9 (50.0%)	8 (80%)	56 (73.7%)	
Age (years)	mean (SD)	57.6 (12.9)	56.9 (14.4)	55.9 (9.4)	58.0 (18.4)	58.8 (12.2)	F=0.8, p=0.517
Total measures	mean (SD)	26.4 (17.4)	24.5 (12.6)	23.8 (11.5)	21.8 (10.4)	26.4 (17.4)	F=0.4, p=0.755
Valid measures	mean (SD)	19.8 (10.8)	20.3 (12.0)	18.3 (8.4)	20.5 (10.8)	19.9 (11.1)	F=0.1, p=0.933
Exam duration (sec)	mean (SD)	182 (270)	251 (475)	164 (135)	114 (121)	171 (194)	F=0.9, p=0.453
TE kPa,median mean (SD) [IQR]		6.3 (4.5) [2.4]	5.2 (3.1)	5.7 (5.3)	5.1 (1.6)	7.0 (4.8)	F=1.6, p=0.195
Cut Off ≤7.0 kPa	n=102 (77%)	4.6 (1.1)	4.1 (0.9)	4.2 (1.2)	4.7 (0.9)	4.9 (1.0)	F=4.1, p<0.001
7 <kPas≤ 9	n=12 (9%)	7.9 (0.6)	7.7 **	7.6 **	8.8 **	7.8 (0.6)	F=0.9, p=0.478
9< kPa ≤10.5	n=3 (2%)	10.3 (0.2)	10.4 **	–	–	10.2 (0.3)	F=0.3, p=0.667
≥10.5 kPa	n=15(12%)	15.8 (7.4)	14.4 (0.6)	26.3 **	–	15.1 (7.7)	F=1.1, p=0.366
TE kPa IQR	mean (SD)	1.3 (2.4)	1.0 (0.8)	1.1 (1.2)	0.9 (0.5)	1.5 (3.1)	F=0.4, p=0.735
TE kPa IQR /TE kPa median (%)	mean (SD)	18.0 (10.6)	9.0 (1.7)	20.1 (17.3)	16.9 (8.1)	17.3 (9.5)	F=0.5, p=0.691
CAP median (dB/m)	mean (SD)	288 (60.8)	205 (53.2)	259 (5.0)	274 (3.6)	328 (32.3)	F=80.3, p<0.001
CAP IQR (dB/m)	mean (SD)	33.3 (18.1)	43.2 (25.7)	33.6 (15.6)	35.1 (14.5)	29.3 (14.0)	F=4.4, p=0.006
CAP IQR (dB/m) Cut Off >40 (n=36, i.e. 27% of NAFLD)	mean (SD)	55.0 (18.7)	60.9 (24.6)	52.4 (12.5)	51.7 (12.7)	50.7 (14.2)	F=0.8, p=0.529

Disclosure of Interest: None Declared

P01-07

Factor influencing ainotransferase activity reduction due to lifestyle modification in children with nonalcoholic fatty liver disease

Anna Mania[*] [1], Pawel G. Malecki[1], Pawel Kemnitz[1], Katarzyna Mazur-Melewska[1], Wojciech Sluzewski[1], Magdalena Figlerowicz[1]

[1]Department of Infectious Diseases and Child Neurology, University of Medical Sciences in Poznań, Poznań, Poland

Corresponding author's email: maniaanna@hotmail.com

Introduction: Sedentary life style and improper eating habits is the most common cause of nonalcoholic fatty liver disease (NAFLD). Children develop fatty liver along with insulin resistance and metabolic syndrome.

Aims: The aim of the study was to analyse clinical factors influencing the course of nonalcoholic fatty liver disease in children

Material and Methods: Forty five children were enrolled in the study, age range 3-17 years (mean 10, 20±3, 41), 29 boys and 16 girls. NAFLD was diagnosed on the basis of abdominal ultrasound examination. Differential diagnosis of the liver steatosis was performed to exclude underlying liver diseases. All patients underwent weight and height measurements, physical examination and blood collection for hematologic and biochemical parameters. Patients received lifestyle modification advices regarding diet and physical exercises. All children were followed during control visits. End-point parameters were reduction of aminotransferase activity and body weight.

Results: 20/45 children had increased BMI, in 9/45 cases weight reduction was observed. Elevated liver enzymes 2xUNL were present in 22/45 patients; in 14 patients a decline in aminotransferase activity was detected. No decline in aminotransferase activity was observed more often in the group with low BMI (0.50±0.89vs -0.19±0.37; p=0.012). In children with reduction of aminotransferase activity higher initial values of AST (52.72±30.79 vs 30.09±12.43 IU/l, p=0.013) and APRI (0.56±0.40 vs 0.26±0.12; p= 0.006) were observed. Patents with a decline in aminotransferase activity had significantly lower initial fasting glucose levels (4.73±0.25 vs 5.21±0.08 IU/l, p=0.036.

Conclusions: Despite attempts of lifestyle modification, weight reduction is not a common effect in children with NAFLD. Children with NAFLD and normal body weight are less likely to achieve a reduction in aminotransferase activity due to lifestyle modification. Liver injury expressed as AST activity does not exclude significant reduction of aminotransferase after lifestyle modification.

Disclosure of Interest: None Declared

P01-08YI

The peculiarities of lipid compound of the inner mitochondrial membrane of hepatocytes due to glutamate-induced steatosis and variants of its correction

Antonina Antonenko[*] [1], Tatyana Beregova[2], Liudmyla Ostapchenko[3], Tetyana Falalyeyeva[2], Daryna Voieikova[2]

[1]Internal Medicine №3, Bogomolets National Medical University, [2]Taras Shevchenko National University of Kyiv, [3]Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

Corresponding author's email: antoninaantonenko2@gmail.com

Introduction: Steatosis or non-alcoholic fatty liver disease (NAFLD) is considered one of the most common forms of chronic liver disease for today. Among main factors of steatosis are the disruption of diet and physical inactivity. In obese individuals lipid metabolism is impaired, which leads to excessive fat accumulation in the body. Monosodium glutamate is widespread food supplement that leads to obesity and steatosis. The derivate of thiazolidinedione (pioglitazone) was studied as effective hypoglycaemic drug with improving of steatosis. Recently antioxidant effects of cerium dioxide nanoparticles (nCeO2), a type of engineered nanomaterials, have been reported

Aims: The aim of this work is to determine the lipid compound of the inner mitochondrial membrane of hepatocytes and to determine the ways of its correction.

Material and Methods: 40 white male Wistar rats were divided into 4 groups: control, monosodium glutamate (MSG)-induced obesit, MSG treated with pioglitazone (MSG+pioglitazone) and MSG treated with nCeO2 (MSG+nCeO2) groups. Newborn rats of control group were injected with saline (control). MSG- and MSG+nCeO2 groups were injected with MSG (4 mg/g concentration, 8 mcl/g volume) between the 2nd and the 10th days of life subcutaneously. The division of phospholipids was done with two-dimensional microthinlayer chromatography with the use of plates Sorbfil (10x10 cm). The determination of total and free cholesterol was based on the reaction of interaction of coloured reagent with cholesterol in different temperature conditions.

Results: In control group the level of phospholipids was 218,65±10,93 mkg/mg of protein that was 1,5 times less than in MSG-induced group ($p<0,05$). In the case of correction of stetosis the increase of phospholipids was observed in 1,2 times ($p<0,05$). The level of cholesterol was decreased in 2 times (MSG+nCeO2) and 1,5 times (MSG+nCeO2), $p<0,05$. In 4-month rats we found significantly lower total score (1.3±0.26 vs 3.6±0.34, $p<0.001$), degree of steatosis (1.1±0.18 vs 2.1±0.18, $p<0.001$), manifestation of lobular inflammation (0.2±0.13 vs 1.2±0.2, $p<0.001$) and ballooning degeneration (0.0±0.0 vs 0.3±0.15, $p=0.034$) due to NAS in the nCeO2 group compared to the MSG-group.

Conclusions: Due to its antioxidant properties nCeO2 significantly reduces the incidence of NASH and improves the main histological features. The use pioglitazone and nCeO2 showed a positive influence on the lipid compound of the inner mitochondrial membrane of hepatocytes.

Disclosure of Interest: None Declared

P01-09YI

African fruits and vegetable juice formulation possesses chemopreventive and chemotherapeutic effects on concanavalin a- model hepatitis in mice

Olufemi E. Akanni[1], Ayodeji O. Faremi[*2] on behalf of Biomedical Research Group, Olusegun H. Ojokuku[3]

[1]Haematology Division, Department of Medical Laboratory Science, Ladoke Akintola University of Technology, Ogbomosho, [2]Laboratory services, Government Hospital/Ladoke Akintola University of Technology, Ede/Ogbomosho, [3]Research Laboratory, Equitable Health Access Initiative, Akure, Nigeria

Corresponding author's email: ayodejiseun29@gmail.com

Introduction: The patronage for alternative therapies against chemotherapy to cure liver related diseases is increasing in Sub-Saharan Africa due to its readily availability, reduced cost and serious side effects of conventional drugs. Thus identification of alternative therapeutic and potential biological active ingredients are sorely needed.

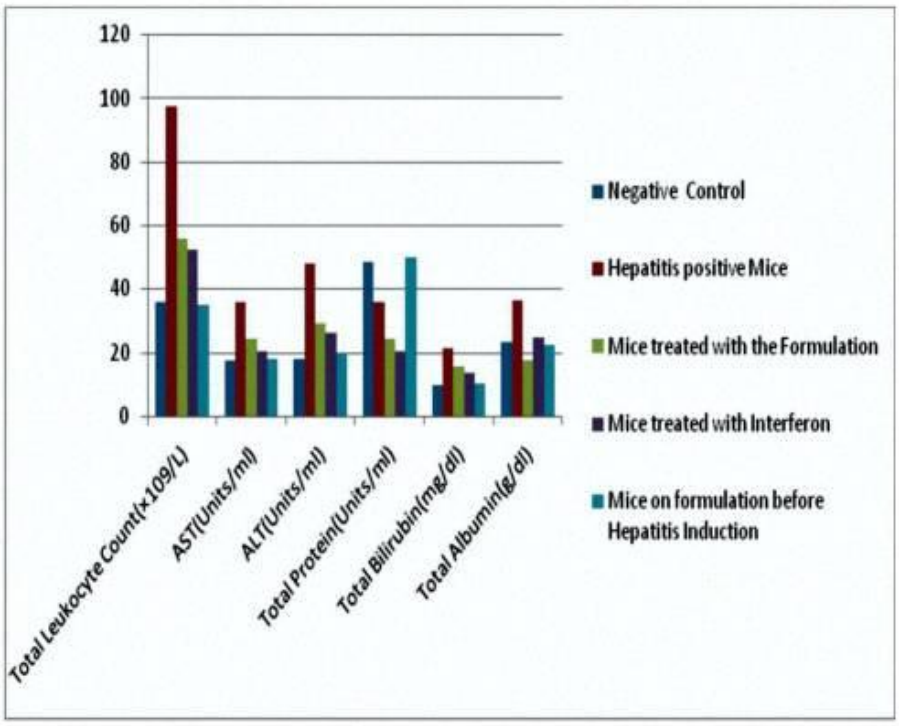
Aims: The aim of the study is to investigate the chemopreventive and chemotherapeutic effects of some fruits and vegetable juice formulation suspended in honey medium on liver marker's parameters and total leucocyte count of concanavalin A induced animal model hepatitis in mice. The formulation is composed of juice of different Nigerian fruits and vegetables which are *Telfairia occidentalis, Citrus sinensis and seeds, Citrus aurantifolia and seeds, Citrus lemon and seeds, Vitis vinifera and seeds, Ananas comosus and peel*.

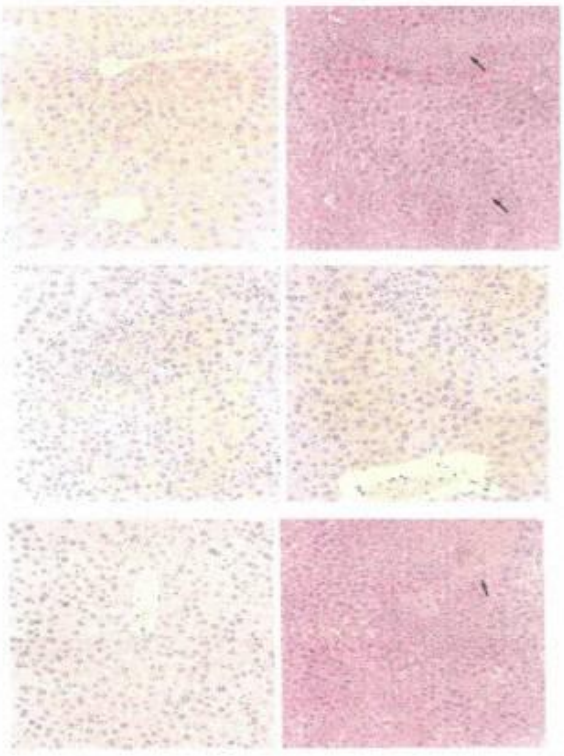
Material and Methods: Hepatitis was induced by injecting 0.4ml of concanavalin A solution intravenously through the tail of the mice at 24h and 48 h intervals. Hepatic injuries and the effect of the formulation were assessed using indicator parameters such as total leukocyte count and liver enzyme makers (serum AST, ALT, Total protein, Albumin, serum bilirubin). Following acute toxicity studies 0.41 ml of the formulation was administered orally by gavage using oral cannular once daily post hepatitis induction for six weeks.

Results: Hepatitis induction reflected a remarkable significance increase in Total leukocyte count, serum AST, ALT, Total protein, Albumin, serum bilirubin over controls ($P<0.05$). Also significant differences was observed between chemotherapeutic group (hepatitis positive group treated with the formulation) and the hepatitis positive control group untreated. Similarly significance difference was observed between chemopreventive group of mice treated with standard drug (interferon) and mice on the formulation. Moreso, the formulation shows similar anti hepatitis performance with the standard drug both in biochemical and histhopatological studies.

Conclusions: The results revealed the profound activity of the formulation at preventing and ameliorating experimentally induced hepatitis, hence the ability of the product in the treatment of acute liver injury in humans.

Figure:





Disclosure of Interest: None Declared

P01-10YI

Epigenomic profiling of hepatocytes overexpressing the lipogenic and tumor promoting mRNA binding protein p62/IMP2-2

Beate M. Czepukojc[*] [1], Sonja M. Keßler[1], Cristina Cadenas[2], Kathrin Gianmoena[2], Nina Gasparoni[3], Karl Nordström[3], Gilles Gasparoni[3], Maren Falk-Paulsen[4], Anupam Sinha[4], Philip Rosenstiel[4], Jan G. Hengstler[2], Jörn Walter[3], Alexandra K. Kiemer[1]

[1]Department of Pharmacy, Saarland University, Saarbrücken, [2]Systems Toxicology, Leibniz Research Centre for Working Environment and Human Factors (IfADo), Dortmund, [3]Department of Genetics, Saarland University, Saarbrücken, [4]Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, Kiel, Germany

Corresponding author's email: beate.czepukojc@uni-saarland.de

---

Introduction: The insulin-like growth factor 2 (*IGF2*) mRNA binding protein p62/IMP2-2/IGF2BP2-2 was originally identified as an autoantigen in hepatocellular carcinoma (HCC) and was found to induce steatosis in a liver-specific *p62* transgenic mouse model. p62 also promotes other liver pathologies, such as fibrosis, cirrhosis and HCC, most of which were shown to be also relevant for human diseases.

Aims: Previous studies reported that especially the expression of imprinted genes is elevated by *p62*. Therefore, the aim of this study was to investigate a potential role of p62 overexpression on the epigenome of hepatocytes and a potential paracrine action on the epigenome of hepatic non-parenchymal cells (NPCs).

Material and Methods: Hepatocytes and NPCs o 9-week-old wild-type (wt) and *p62* transgenic (tg) mice, expressing the transgene exclusively in hepatocytes, were isolated. Gene expression and DNA methylation was analyzed in both cell types by RNA-seq and reduced representation bisulfite sequencing (RRBS), respectively. In addition, DNase I hypersensitive sites sequencing (DNaseI-seq) data were generated for hepatocytes to identify open chromatin regions.

Results: The RRBS data revealed 674 differentially methylated regions (DMRs) for hepatocytes of *p62* tg mice compared to the wt with a similar amount of hyper- and hypomethylated regions. RNA sequencing revealed 237 differentially expressed genes in *p62* tg hepatocytes. The expression level of the imprinted genes *IGF2* and *H19* was significantly increased, but not associated with epigenetic changes. Among the differentially expressed genes 61 were associated with epigenetic changes, i.e. DMRs and/or differential open chromatin regions (DOR). Interestingly, GO term analysis revealed that these genes are involved in metabolic processes or chemical induced toxicity, such as cellular response to chemical stimuli, lipid metabolic processes, and fatty acid metabolic processes, all of which are involved in NASH-related lipid accumulation and lipotoxicity. In NPCs isolated from *p62* tg mice compared to wt mice 1,888 mostly hypomethylated DMRs were found. However, NPCs of *p62* tg animals had only 42 differentially expressed genes compared to wild-types with 5 genes associated with epigenetic changes.

Conclusions: Differential gene expression associated with epigenetic changes were mostly found in the hepatocytes of *p62* tg mice with minor paracrine effects on NPCs. These genes particularly play a role in metabolic and toxicity processes, both of which are important in NASH pathogenesis.

Disclosure of Interest: None Declared

P02-01YI

Dietary patterns in Irish patients with non-alcoholic fatty liver disease: a cross sectional study

Carla Bredin[* 1], Luya Li[2], Sara Naimimohasses[3], Suzanne Norris[3], J. Bernadette Moore[1, 2], Katherine Hart[4], Ciara Wright[5]

[1]Department of Nutritional Sciences, University of Surrey, Guildford, Surrey, [2]School of Food Science and Nutrition, University of Leeds, Leeds, United Kingdom, [3]Department of Hepatology, St James's Hospital, Dublin, Ireland, [4]Department of Nutritional Sciences, University of Surrey, Guidlford, Surrey, United Kingdom, [5]Glenville Nutrition, Dublin, Ireland

Corresponding author's email: carlabredin@yahoo.ie

Introduction: Non-alcoholic fatty liver disease (NAFLD) is now the most common form of liver disease in the developed world, but has been understudied in Ireland. Although the etiology of NAFLD is complex, it is well accepted that excess energy consumption and poor diet quality contribute to its development and progression.

Aims: The aim of this study was to characterise the dietary intakes of Irish adults with a diagnosis of NAFLD attending a large tertiary referral site.

Material and Methods: NAFLD patients, diagnosed by liver ultrasound and Fibroscan imaging, were recruited from St James' Hospital in Dublin, Ireland. Consenting patients underwent a 20-minute interview involving a short (48-item), food frequency questionnaire (SFFQ) developed specifically to assess habitual intakes of food items related to NAFLD. In addition, patients filled out a 4-Day Diet Diary (4DDD) at home that was returned by mail. Nutrient intakes were analysed using myFood24 dietary analysis software and assessed in comparison to the recommended dietary allowances (RDA) for Ireland

Results: To date, 67 patients have consented and completed the SFFQ, 43 of whom have also completed the 4DDD. Participants were more frequently male (61%), middle-aged (54.0 ± 11.5 years) and obese, with a mean [95% CI] BMI of 32.8 [31.3, 33.5]. Initial dietary analyses (n=13 4DDD) showed intakes of total energy and total fat within the RDA, but intakes of free sugars (231% and 204% RDA for men and women respectively) and dietary protein (148% and 167%) greatly exceeded recommendations. Sodium intakes also exceeded the RDA (138% and 125%), and, to a lesser extent, saturated fat (102% and 111%). Participants did not meet recommended intakes for dietary fibre (76% and 80%); and vitamin D intakes were markedly below recommendations (21% and 24%). In general, most participants (n=33 measured) had insufficient circulating 25-hydroxyvitamin D levels with mean [95% CI] levels of 45.4 nmol/L [37.4, 53.5].

Conclusions: This is the first study to examine diet and nutrient intakes in an Irish NAFLD population, and data collection and analyses are ongoing. Participants consumed excess free sugars, protein and sodium, and had sub-optimal intakes of fibre. In line with other reports of widespread vitamin D insufficiency in Ireland, our patients consumed very low amounts and had insufficient circulating levels of vitamin D. These results support current recommendations for vitamin D supplementation in Ireland

Disclosure of Interest: None Declared

P02-02

Dual CCR2-5 antagonist decreases hepatic inflammation in acute liver injury and NASH metabolic animal models

Trent T. Ross[1], Marc Roy [2], Tim Coskran[2], David A. Beebe[1], Collin Crowley[1], Derek Erion[1], Julie Purkal[1], William Esler[1], Kenneth Kelly[1], Jessie Qian[1], Jeffrey Pfefferkorn[1], Aditi Saxena[1], Cecile Vernochet[*1]

[1]Internal Medicine, Pfizer, Cambridge, [2]DSRD, Pfizer, Groton, United States

Corresponding author's email: cecile.vernochet@pfizer.com

Introduction: The chemokine receptor types 2 and 5 (CCR2 and CCR5) are hypothesized to play a central role in hepatic inflammation and fibrogenesis contributing to the pathogenesis of NASH.

Aims: We evaluated CCR2, CCR5 and their corresponding activating chemokines such as CCL2, CCL3, CCL4 and CCL4 expression from NAFLD/NASH confirmed human liver biopsies. Impact of oral administration of a CCR2/CCR5 antagonist was evaluated in a NAFLD/NASH and liver injury model.

Material and Methods: Impact of oral administration of a CCR2/CCR5 antagonist was evaluated in a metabolic driven NASH rodent model "Streptozotocin and high fat diet- induced NASH" (STAM™) mouse and in thioacetamide (TAA) treated male C57BL6 mice, a hepatotoxicant mediated inflammation and liver injury model.

Results: CCR2, CCR5 and CCL2 were particularly upregulated when fibrosis was detected in human liver biopsies. The aggregate NAS score was significantly reduced by the CCR2/CCR5 antagonist administration compared to vehicle treated STAM™ mouse and to a similar extend compared to OCA treated animal group. CCR2/CCR5 antagonist administration significantly suppressed elevations in ALT and AST levels elevation upon acute administration of TAA to rodents. CCR2/CCR5 antagonist decreased circulating pro-inflammatory and total monocyte levels. TAA induced liver damage and associated inflammation resulted in significant expression changes in 81/248 genes in the mouse inflammation panel, relative to the vehicle control group as addressed using Nanostring Mouse Inflammation panel. CCR2/5 antagonist administration reduced the number differentially expressed genes by 47 (34/248) including complement component 4a and 9 (C4a and C9). Effects of TAA upon hepatic expression of genes associated with inflammation were nearly completely reversed in a set of 15 genes, a majority of which are associated with the acute phase response and IL-6 signaling pathways.

Conclusions: Taken together, these data indicate that CCR2/5 axis is upregulated in human liver NASH and suggest that administration of the CCR2/CCR5 antagonist produced an anti-inflammatory response in both a chemotoxic mediated inflammation and liver injury model as well as in a metabolically driven inflammation model.

Disclosure of Interest: T. T. Ross: Employee: Pfizer, M. Roy: Employee: Pfizer, T. Coskran: Employee: Pfizer, D. A. Beebe: Employee: Pfizer, C. Crowley: Employee: pfizer, D. Erion: Employee: pfizer, J. Purkal: Employee: Pfizer, W. Esler: Employee: Pfizer, K. Kelly: Employee: Pfizer, J. Qian: Employee: Pfizer, J. Pfefferkorn: Employee: Pfizer, A. Saxena: Employee: Pfizer, C. Vernochet: Employee: Pfizer

P02-03

Chronic mild stress attenuates hepatic steatosis and inflammation in a high fat-diet fed-mouse model

Chang Gue Son*[1], Hyeong Geug Kim[1], Sung Bae Lee[1], Jin Seok Lee[1], Won Yong Kim[1], Myong Min Lee[1]

[1]Daejeon Oriental Hospital of Daejeon University, Daejeon, Korea, South

Corresponding author's email: ckson@dju.ac.kr

Introduction: Nonalcoholic fatty liver disease (NAFLD) is major cause of chronic liver disorders and characterized by more 5% accumulation of hepatic triglyceride. NALFD encompasses a broad clinic pathological spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH). Meanwhile, stress has been proposed as a risk factor in the development and progression of diverse diseases including metabolic syndrome and cardiovascular disease.

Aims: Recently, studies for relationship between enviroment factor (exercise, alcohol, etc.) and NAFLD have been actively conducted. However, relation of stress NAFLD has not proved. In this study, we investigated the influence of chronic mild stress on HFD-induced NAFLD, and its underlying mechanisms using a mouse model.

Material and Methods: We evaluated the effects of mild stress, which did not damage to the liver, on nonalcoholic fatty liver disease (NAFLD) in a high-fat diet (HFD)-fed mouse model. C57/BL6 mice had free access to a 60% HFD for 8 weeks, with or without repeated restraint stress (3 h) conducted 3 times a week.

Results: HFD administration substantially increased fat accumulation overall and in hepatic tissues as shown by Oil Red O staining and measurement of hepatic total cholesterol (TC) and triglyceride (TG). Stress alone showed a tendency to increase hepatic TC and serum TG compared to naive group, while the HFD plus stress group had significantly improved hepatic TC and TG levels compared to the HFD group. These beneficial results were in accordance with serum levels of liver enzymes (AST; aspartate transaminase and ALT; alanine transaminase), inflammatory cytokine (TNF-α; tumor necrosis factor alpha) and oxidative stress parameters including reactive oxygen species (ROS), nitric oxide (NO), and malondialdehyde (MDA). The HFD-induced imbalance between lipogenesis and lipolysis was significantly improved by repeated mild restraint stress as determined by gene expression analyses.

Conclusions: Our study first presented the beneficial effects of chronic mild stress on hepatic steatosis and its related injury. However we need further studies to confirm these results using various levels or types of stress. In conclusion, predictable mild stress has a preventive effect on high fat intake-associated hepatic steatosis and its progression into NASH. The underlying mechanisms involve the regulation of triglyceride synthesis and accumulation in hepatic tissue, and the oxidative stress-related hepatic inflammatory response.

Disclosure of Interest: None Declared

P02-04YI

Comparing the role of hepatocyte-specific knockout of Basigin in mouse models of liver injury and effect on extracellular vesicle production in NAFLD

Christine Yee[*1,2], Linda Ban[3], Prudence Wignall[1], William d'Avigdor[1], Alexandra Terry[1], Susan McLennan[3], Nicholas Shackel[2,4,5]

[1]Liver Cell Biology, Centenary Institute of Cancer Medicine and Cell Biology, Camperdown, [2]Gastroenterology and Liver Laboratory, Ingham Institute of Applied Medical Research, Liverpool, [3]Sydney Medical School, The University of Sydney, [4]Faculty of Medicine, University of New South Wales, Sydney, [5]South West Sydney Clinical School, University of New South Wales, Liverpool, Australia

Corresponding author's email: c.yee@centenary.org.au

Introduction: Basigin (or CD147) is a multifunctional, highly glycosylated transmembrane protein that has been implicated in fibrosis development due to its roles in matrix metalloproteinase (MMP) induction and chemotaxis. Global Basigin-knockout mice and anti-Basigin antibody interventions with liver injury has been shown to significantly decrease liver fibrogenesis. Furthermore, Basigin secreted from extracellular vesicles (EV) have been shown to influence liver disease progression via hepatic stellate cell activation.

Aims: As the role of Basigin on hepatocytes in liver injury and NAFLD is not clear, this study aims to investigate the contribution of hepatocyte-specific Basigin in two liver injury models: 1) induction by CCl_4 for modelling progressive liver injury and its effect on fibrosis and 2) high-fat feeding model of NAFLD and its effect on EV production.

Material and Methods: Eight-week old, male Basigin$^{fl/fl}$ x AlbCre^{+} (hepatocyte-specific) knockout or Basigin$^{fl/fl}$ Cre^{-} (control) mice were terminated 72 hours after a single *i.p.*dose of 12% carbon tetrachloride (CCl_4) in paraffin oil or oil alone, or after twice weekly doses for 4 or 8 weeks. For high-fat feeding model of NAFLD, mice were fed a high-fat diet (HFD, 45%kcal from fat, 0.5% cholesterol) or chow diet (12%kcal fat) *ad libitum* for 12 weeks. Serum was analysed for liver transaminases. For high-fat fed mice, extracellular vesicles (EVs) were taken from supernatant of cultured liver tissue and plasma, with particles concentration determined via Nanosight. Plasma triglycerides and hepatic lipid were also measured.

Results: Knockout mice are healthy, viable and exhibit no off-target effects compared to control. Hepatocyte-specific Basigin-knockout mice did not differ from control in body weight and liver weight in CCl_4-treated mice, as with HFD-fed mice. During acute liver injury with CCl_4 (72hrs), ALT and AST were decreased 3-4 fold ($p<0.01$) in knockout mice, whereas no difference in serum transaminases was observed for HFD-fed mice. Deviations in liver lipid did not reach significance, and total EV numbers were not changed in the absence of hepatocyte Basigin, for either plasma or liver EVs.

Conclusions: Our data suggests that hepatocyte-derived Basigin is important in the initiation of fibrogenesis, as demonstrated in a chemically-induced liver injury model. In contrast, absence of Basigin on hepatocytes does not contribute to changes in EV concentration in the plasma or from liver tissue, irrespective of diet-induced NAFLD.

Disclosure of Interest: None Declared

P02-05

AXA1125, a novel defined amino acid composition (DAAC), improves NAFLD activity score (NAS) and reduces fibrosis in two rodent models of nonalcoholic steatohepatitis (NASH)

Chung-Wei Lee[*1], Raffi Afeyan[1], Harry Luithardt[1], Michael Hamill[1], Manu Chakravarthy[1], Tony Tramontin[1]

[1]Axcella Health, Cambridge, United States

Corresponding author's email: cwlee@axcellahealth.com

Introduction: AXA1125 is a defined amino acid composition (DAAC™) designed to simultaneously target multiple mechanisms of disease pathology to safely and effectively treat NASH.

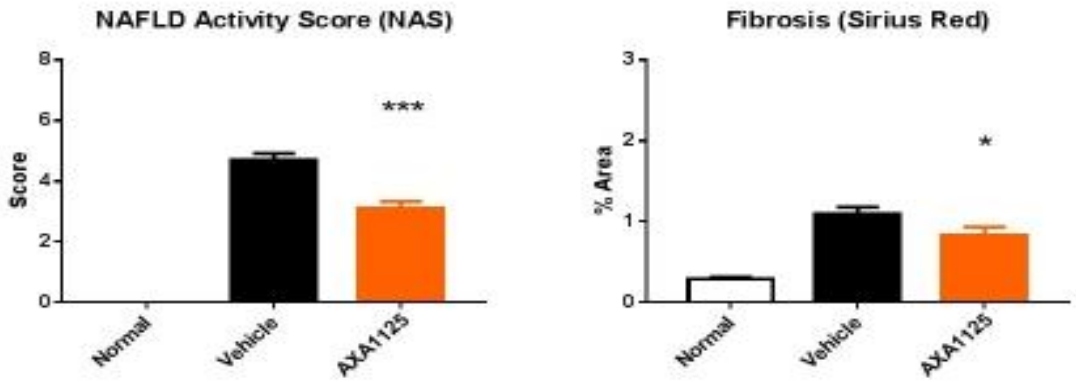
Aims: Here, we studied the effects of AXA1125 in NASH using two established NASH mouse models.

Material and Methods: STAM model (Stelic MC, Inc.) was created in male C57BL/6J mice that received neonatal streptozotocin treatment. A high fat diet (HFD) containing 56.7% kcal fat was provided ad libitum staring from 4 weeks of age. FATZO mice (Crown Biosciences, Inc.) were fed with a high fat, fructose, and cholesterol diet (HFFC) containing 40% kcal fat ad libitum. AXA1125 was administered orally for 3 weeks in STAM mice (6 to 9 weeks old), and for 4 weeks in FATZO mice (16 to 20 weeks post diet induction). Body weight and food intake were recorded. Liver histology, lipids, gene expression and metabolites were evaluated.

Results: Treatment with AXA1125 effectively reduced NAFLD activity scores (NAS) in both STAM and FATZO mice. AXA1125 decreased hepatocyte ballooning score and fibrosis area in both models. Scores of steatosis and inflammation and liver triglyceride levels were not changed according to histological measures. However, in the STAM model, liver gene expression pattern of AXA1125 overlapped with pathway of enhanced peroxisomal fatty acid beta-oxidation, and this was confirmed by lowered unsaturated fatty acids and higher acylcarnitines in the liver of AXA1125 treated mice. Liver gene expression pattern of AXA1125 also overlapped with pathways of upregulated anti-inflammatory IL-10 and downregulated pro-inflammatory NF-kB, interferons, IL-1b and IL-2. At the protein level, AXA1125 significantly down-regulated hepatic MCP-1 and MIP-1. Additionally, liver gene expression pattern of AXA1125 overlapped with reduced fibrogenic TGF-beta pathway activity.

Conclusions: AXA1125 ameliorates NASH progression in two rodent models by impacting lipid metabolism, inflammation, and fibrosis.

Figure:



Disclosure of Interest: C.-W. Lee: Employee: Axcella Health, R. Afeyan: Employee: Axcella Health, H. Luithardt: Employee: Axcella Health, M. Hamill: Employee: Axcella Health, M. Chakravarthy: Employee: Axcella Health, T. Tramontin: Employee: Axcella Health

P02-06YI

Network based drug repositioning for Non-Alcoholic Fatty Liver Disease

Danae S. Zareifi[*] [1], Nafsika Chala[1], Dimitris Messinis[1], Asier Antonaz[1], Angeliki Minia[2], Vaia Pliaka[2], Jan Rozanc[2], Manoussos M. Konstadoulakis[3], Konstantinos J. Bramis[3], Efstathios A. Antoniou[4], Eirini Pantiora[5], Andreas Polydorou[5], Antonios Vezakis[5], Georgios Fragulidis[5], Leonidas G. Alexopoulos[1, 2]

[1]Mechanical Engineering, National Technical University of Athens, [2]ProtATOnce Ltd., [3]1st Department of Propaedeutic Surgery, Hippokration General Hospital, Medical School, University of Athens, [4]2nd Department of Propaedeutic Surgery, Laiko Hospital, University of Athens, School of Medicine, [5]2nd Department of Propaedeutic Surgery, Aretaieio Hospital, University of Athens, School of Medicine , Athens, Greece

Corresponding author's email: danae.zar@gmail.com

Introduction: Non-alcoholic fatty liver disease (NAFLD) is the most common pathological condition of the liver. Many pharmacological agents have been tested for the management of the disease, but there is no therapy approved specifically for NAFLD by the US Food and Drug Administration. On this front, drug repositioning (DR) offers an accelerated route for drug discovery.

Aims: The aim of this project is to suggest a platform for drug repositioning in NAFLD by combining novel *in vitro* models of primary human hepatocytes with network-based analysis of gene expression data from NAFLD patients.

Material and Methods: To induce NAFLD *in vitro*, primary human hepatocytes were exposed to free fatty acids (FFAs, palmitic and oleic acid) and to the steatogenic compounds amiodarone (AMI), tamoxifen (TMX), tetracycline (TET) and valproic acid (VPA). The formation of intracellular lipid droplets was verified using high content screening; lipid droplets were stained with Nile Red fluorescent probe, Hoechst 33342 was used for staining cell nucleus. The intracellular ROS production was measured using the fluorescent substrate CM-H_2DCFDA. A network-based computational approach was employed to suggest compounds for NAFLD. Briefly, gene expression networks derived from NAFLD patients were matched with drug-induced networks in an effort to identify drugs that affect the NAFLD-mechanisms. NAFLD-related networks were identified through gene set analysis (GSA) of two microarray datasets from GEO-NCBI. Common pathways with the steatogenic compounds used to induce NAFLD *in vitro* were found through Drugbank and MSig databases. To suggest compounds that reverse the disease mechanism, the steatogenic compounds were used with the Connectivity Map database. The promising compounds for DR are considered to belong in the intersection of GSA-derived and drug-derived networks.

Results: Lipid droplet accumulation and ROS production was present in all *in vitro* models, as it is observed in patients with NAFLD. The *in silico* analysis for DR identified Naftifine, Pralidoxime, Fusidic acid, Raloxifene, Oxprenolol, Dipivrefin, Metoprolol, Estrone sulfate, Physostigmine, Cefmetazole and Diflorasone as promising compounds for NAFLD treatment.

Conclusions: We have successfully developed NAFLD-induced *in vitro* models using the steatogenic compounds FFAs, VPA, TMX, TET and AMI. We have identified mechanisms of NAFLD pathogenesis through gene set analysis. The efficacy of the resulting compounds from the DR platform is being tested on the NAFLD *in vitro* models.

Figure:

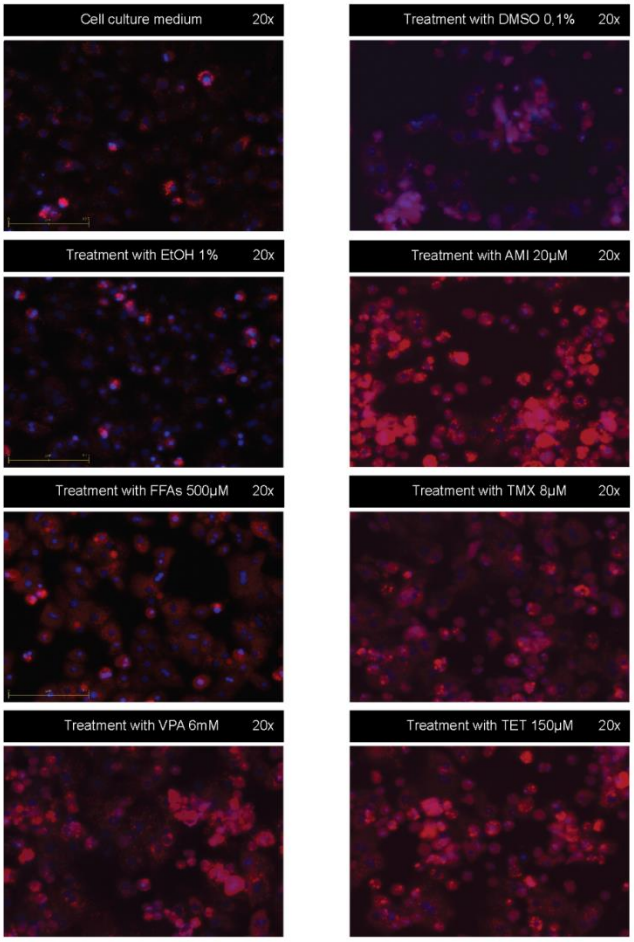


Figure 1. Intracellular lipid droplet accumulation after 24h exposure of primary human hepatocytes to Free Fatty Acids (FFAs; oleic and plamitic acid) and to the steatogenic compounds amiodarone (AMI), tamoxifen (TMX), tetracycline (TET) and valproic acid (VPA). Lipid droplets were stained with Nile Red fluorescent probe and Hoechst 33342 was used for counterstaining cell nucleus. Images were acquired by JuLI™ Stage Real-Time CHR (Cell History Recorder) (NanoEnTek) under 20x optical magnification.

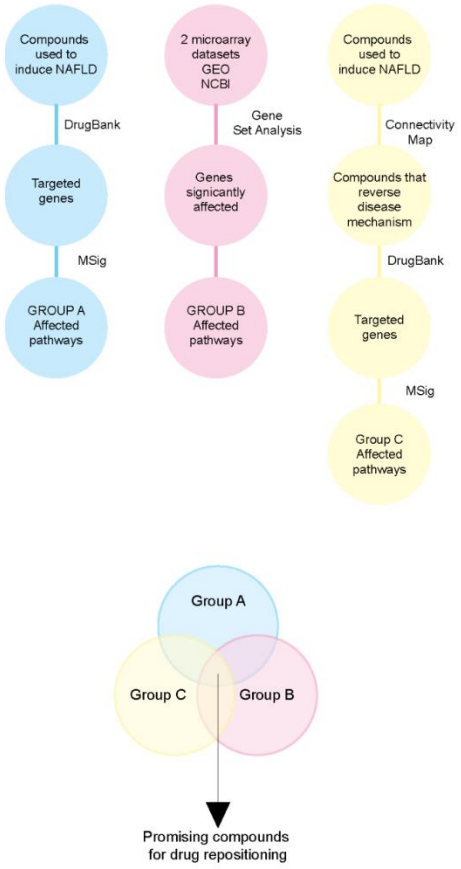


Figure 3. Workflow for the drug repositioning platform for NAFLD/NASH. NAFLD-related networks were identified through gene set analysis (GSA) of two microarray datasets of biopsy proven NAFLD/NASH patients from GEO (NCBI) (Group B). Pathways affected by the steatogenic compounds used to induce NAFLD *in vitro* were found through Drugbank and MSig databases (Group A). The steatogenic compounds were used with the Connectivity Map database to suggest compounds that reverse the disease mechanism (Group C). The most promising compounds for drug repositioning are considered to belong in the intersection of Groups A, B and C.

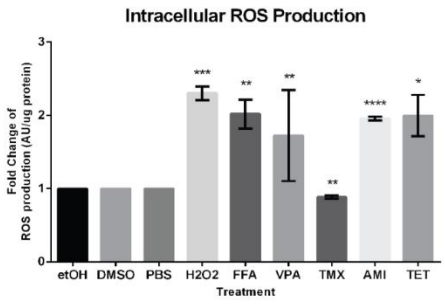


Figure 2. Fold change of ntracellular ROS production after 24h exposure of primary human hepatocytes to Free Fatty Acids (FFAs; oleic and plamitic acid) and to the steatogenic compounds amiodarone (AMI), tamoxifen (TMX), tetracycline (TET) and valproic acid (VPA). The intracellular ROS production was measured using the fluorescent substrate CM-H2DCFDA. Fluorescence was measured using Varioskan™ LUX multimode microplate reader (Thermo Scientific™) an normalized by µg of protein. Treatment with H2O2 was used as positive control. Data are expressed as mean±SEM of three independent experiments.

Disclosure of Interest: None Declared

P02-07

Decreased HO-1 expression and reduced mitochondrial function contribute to the development of NASH in obese mice

David Sacerdoti[* 1, 2], Shailendra P. Singh[2], Rita Rezzani[3], Luigi F. Rodella[3], David Bamshad [2], Nader G. Abraham[2]

[1]Medicine, University of Padova, Padova, Italy, [2]Pharmacology, New York Medical College, Valhalla, New York, United States, [3]Pathology, University of Brescia, Brescia, Italy

Corresponding author's email: david.sacerdoti@unipd.it

Introduction: Non-alcoholic steatohepatitis (NASH) is the consequence of insulin resistance, fatty acid accumulation, oxidative stress and lipotoxicity. Increased heme-oxygenase-1 (HO-1) expression acts as an anti-inflammatory signaling protein and an amplifier of antioxidant ability, in addition to its distinct function of recycling iron.

Aims: We hypothesize that a decrease of HO-1 increases hepatic heme-derived oxidative stress, decreases mitochondrial integrity, and contributes to NASH in obese mice.

Material and Methods: To test this, mice were fed a HF diet and obese animals were administered either cobalt protoporphyrin (CoPP) to induce HO-1 or CoPP + stannic mesoporphyrin (SnMP) to attenuate the HO-1 induction caused by CoPP.

Results: NASH mice displayed increased heme levels, and decreased HO-1 and PGC-1α, with increased FAS, NOV, and hepcidin. Mitochondrial fusion genes Mfn1/2, OPA1 decreased while fission Fis1 and Drp1 genes increased, decreasing mitochondrial function. Thermogenic protein SIRT3, the target of PGC-1α, decreased as well as MnSOD. An increase of HO-1 increased ferritin, decreased hepatic heme, lipid deposits, inflammation and fibrosis. In addition, increased levels of HO-1 improved hepatic mitochondrial integrity with increased fusion proteins. In adipose tissue, increased HO-1 levels were associated with a decrease in inflammed large adipocytes, but an increase in small adipocytes as well as adiponectin secretion, and PGC-1α. These effects suggest that HO-1 induction causes a phenotypical change from white fat to brite fat.

Conclusions: In conclusion, the development and progression of obesity-induced NASH may be attenuated by increased levels of HO-1 which would make it a novel potential treatment for this component of the metabolic syndrome, rescuing the liver from lipotoxicity, restoring adipose tissue insulin sensitivity, and preventing activation of inflammatory pathways and oxidative stress.

Disclosure of Interest: None Declared

P02-08

Pooled analysis of saroglitazar PRESS trials - Potential benefits in NAFLD / NASH

Deven V. Parmar[1], Jayesh K. Bhatt[1], Krupi Parmar[1], Mukul Jain[*1], Rajendra H. Jani[1]

[1]Cadila Healthcare Limited, Ahmedabad, India

Corresponding author's email: deven.parmar@zyduscadila.com

Introduction: Saroglitazar is a dual PPARα/γ agonist with predominant PPAR alpha agonist activity, approved in India for the treatment of diabetic dyslipidemia. In nonclinical NASH models, Saroglitazar lowered liver dysfunction markers and histologically showed suppression of steatosis, ballooning, inflammation and fibrosis, indicating potential benefit in fatty liver diseases.

Aims: To assess the trends of liver enzymes, as non-invasive markers of NAFLD/NASH, from the data of various Prospective Randomized Efficacy & Safety of Saroglitazar (PRESS) Trials.

Material and Methods: Data from nine Phase-II & III clinical studies of Saroglitazar were pooled, which included dyslipidemic (TG ≥200 mg/dL) patients with and without type 2 diabetes mellitus. Changes in biomarkers of dyslipidemia, diabetes mellitus as well as liver function test were compared (baseline to week 12) using pair t-test.

Results: Data included total 543 patients with a mean age of 48.73±10.18 years and mean BMI of 26.61±4.09 kg/m^2. These patients were randomized to receive Saroglitazar (0.5, 1.0, 2 and 4 mg). Pooled data analysis showed statistically significant improvements in ALT, a sensitive biomarkers of NAFLD/NASH. Saroglitazar treatment also showed statistically significant improvements in lipid profile and fasting plasma glucose levels.

Conclusions: Saroglitazar, a drug approved in India for treatment of diabetic dyslipidemia shows beneficial effects on lipid & liver enzymes and may offer an effective treatment option for the patients with NASH. Based on these observations, pivotal studies in NAFLD/NASH patients are ongoing in US and India.

Figure:

Table 1: Summary of change from baseline to Week 12					
Parameters	**Time point**	**Saroglitazar**			
		0.5 mg (N=41)	**1 mg (N=45)**	**2 mg (N=179)**	**4 mg (N=278)**
ALT (U/L)	Baseline	29.2±16.1	30.6±19.48	29.6±15.9	28.2±15.2
	Wk12	28.9±19.3	26.7±13.8	25.2±12.5*	23.2±11.9*
AST (U/L)	Baseline	26.1±13.0	27.3±13.4	25.3±12.0	30.3±19.9
	Wk12	27.2±15.8	26.3±11.0	25.9±12.5	29.9±18.3
ALP (U/L)	Baseline	85.7±23.1	88.0±26.0	83.6±25.6	92.1±55.6
	Wk12	72.9±21.3*	65.5±20.6*	65.7±23.5*	58.0±31.5*
GGT	Baseline	30.83±16.17	43.87±34.00	39.50±35.23	57.57±47.95
	Wk12	25.43±15.65	26.83±18.73*	28.55±31.72*	34.90±31.15
HDL (mg/dL)	Baseline	43.3±14.5	46.4±23.8	39.2±14.4	39.7±11.1
	Wk12	43.3±10.7	42.4±14.0	40.4±11.4	42.6±11.6*
TC (mg/dL)	Baseline	200.4±38.8	206.0±39.9	203.5±39.3	199.8±43.0
	Wk12	195.6±41.3	195.8±42.2	172.4±44.9*	174.8±47.0*
TG (mg/dL)	Baseline	241.5±100.5	246.2±121.6	275.0±102.5	271.6±91.8
	Wk12	167.1±71.6*	194.4±199.5*	170.5±86.5*	160.9±83.5*
LDL (mg/dL)	Baseline	95.4±48.6	90.7±41.4	118.8±41.8	120.6±39.9
	Wk12	125.5±38.6	138.9±174.2	99.2±33.0*	106.5±38.8*
FPG (mg/dL)	Baseline	121.3±39.8	124.6±44.4	160.1±64.2	158.9±65.8
	Wk12	116.5±31.6	122.5±44.5	143.5±68.8*	138.6±57.6*

Disclosure of Interest: D. Parmar: Employee: Cadila Healthcare Limited, J. Bhatt: Employee: Cadila Healthcare Limited, K. Parmar: Employee: Cadila Healthcare Limited, M. Jain: Employee: Cadila Healthcare Limited, R. Jani: Employee: Cadila Healthcare Limited

P02-09YI

A comparative analysis of metabolic disturbances in patients with nonalcoholic fatty liver disease, chronic hepatitis C, chronic hepatitis B, alcoholic fatty liver disease and healthy controls

Donika K. Krasteva[*1], Tcvetelina Marinova[1], Radina Ivanova[2], Raya Ivanova[3], Asen Alexiev[1], Dejan Jelev[1], Krasimir Antonov[1], Lyudmila Mateva[1]

[1]Clinic of Gastroenterology, [2]Laboratory of clinical pathology, UMHAT "St. Ivan Rilski", Medical University - Sofia, [3]Clinic of Cardiology, UMHAT "Alexandrovska", Medical University Sofia, Sofia, Bulgaria

Corresponding author's email: donika.krysteva@gmail.com

Introduction: In nonalcoholic fatty liver disease (NAFLD) well known metabolic disturbances as metabolic syndrome (MS), type 2 diabetes mellitus (DM) and insulin resistance (IR). Despite a chronic hepatitis C (CHC) the impact of concomitant hepatic steatosis as well as associated with it metabolic disorders in chronic hepatitis B (CHB) and alcoholic fatty liver disease (AFLD) are not established yet.

Aims: To assess and compare prevalence of conditions associated with the MS, fasting insulin levels and homeostasis model assessment of insulin resistance (HOMA-IR) in patients with CHC, CHB, NAFLD, AFLD with obesity or overweight and healthy controls (HC).

Material and Methods: A total of 1825 subjects were included in this study: 403 NAFLD patients, 550 CHC genotype 1 patients with steatosis (n=330) and without steatosis (n=220), 421 CHB patients with steatosis (n=204) and without steatosis (n=217), 200 AFLD patients with obesity or overweight and 241 HC without fatty liver or other disease, matched for age and gender. Body mass index (BMI), components of MS, serum lipids and serum insulin levels were evaluated.

Results: Higher prevalence and severity of metabolic abnormalities were observed in all patients with steatosis (NAFLD, CHC, CHB and AFLD) than in HC and patients with CHC and CHB without steatosis ($p<0.0001$). Obesity ($p=0.03$) was found to be more prevalent among NAFLD patients and arterial hypertension was found to be more prevalent among NAFLD and AFLD patients than in CHC and CHB patients with steatosis ($p < 0.0001$). There was no significant difference in proportions of overweight, MS, impaired fasting glucose and type 2 DM between groups with steatosis (NAFLD, CHC and CHB with steatosis, AFLD), but HOMA-IR was significantly lower in patients with AFLD ($p=0.01$). The main levels of total cholesterol, low – density lipoprotein (LDL) cholesterol and triglycerides were higher ($p<0.0001$) and the mean levels of high – density lipoprotein (HDL) cholesterol were lower ($p=0.04$) in NAFLD and AFLD cases than CHC and CHB with steatosis, except for total cholesterol and LDL-cholesterol in CHC patients with steatosis.

Conclusions: Metabolic disturbances in NAFLD, NAFLD overlap on CHC and CHB and AFLD patients with obesity or overweight are similar in frequency and degree of deviations.

Disclosure of Interest: None Declared

P02-10

Metabolomics – A new approach to the diagnosis of NAFLD

Igor Bakulin*[1], Liya Belousova[1], Madina Abatsieva[1]

[1]*North-Western state medical University named after Mechnikov, Saint Petersburg, Russia*

Corresponding author's email: igbakulin@yandex.ru

Introduction: Regarding "Omic" technologies, modern achievements to date have been promising. Metabolomics is the youngest science and represents a complete set of low-molecular compounds. Diseases of various organs and systems are accompanied by changes that resulted in the creation in various tissues and biological fluids of certain metabolites that reflect functional changes and serve as biochemical markers. Determination of metabolites allows you to monitor the functional State, track the changes in basic systems, screen pathological changes, as well as identify the effectiveness and efficiency of the therapy.

Aims: to examine data about the potential of "omic" technologies in the diagnosis of NAFLD.

Material and Methods: using databases (Pubmed, Scopus) to analyse the study, which examines the metabolom profile in diseases of the digestive tract.

Results: Currently showing opportunities identification NAFLD potential biomarkers metabolomics, NASH, 2010 study (United States) 437 evaluated various metabolites in serum of patients with NAFLD using ZhH-MS and GC-MS, found that in patients with NASH level of free long-chain fatty acids acids were significantly lower compared to the control group. LoombaR. et al. (2012) explored the importance of polyunsaturated fatty acids as arachidonic, in particular diagnostic biomarkers of non-invasive diagnosis NASH: 11.12-diHETrE, DHK PGD, 20 2-COOH AA were significantly higher in the Group of patients with NASH versus NAFLD. In 2016g. A. Feldman et. demonstrated that NAFLD in patients with BMI ≥ 25 and obese patients have a different profile of amino acids and acyl-carnitine. When NAFLD was given to a group with normal BMI, the result were: lower concentrations of sintez-phosphatidylcholine, alanine, tyrosine and valine and higher lysine concentration in comparison to people with obesity.

Conclusions: assessment of fatty acids as new biomarkers NAFLD and NASH can be used as a differential diagnostic marker and predictor of progressive currents NAFLD in general. In particular, using GC-MS in the determination of serum fatty acids reduces analysis time, increases its accuracy and reliability in various stages of NAFLD whilst helping clinicians with diagnosis and evaluation of prediction.

Disclosure of Interest: None Declared

P03-01

Hepatocellular carcinoma in non-alcoholic steatohepatitis (NASH): Clinical, histopathological aspects and immunohistochemical of metabolic and proliferative related-markers

Priscila B. Campos[1], Claudia P. Oliveira[* 1], José Tadeu Stefano[1], Aline L. Chagas[1], Paulo Herman[1], Luiz C. D'albuquerque[1], Mário R. Alvares da Silva[2], Adhemar Longatto-Filho[3], Fátima Baltazar [3], Sara Granja[3], Flair José Carrilho[1], Venâncio A. Alves[4]

[1]Gastroenterology - LIM 07/37, University of Sao Paulo School of Medicine, Sao Paulo, [2]Gastroenterology, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, [3]Life and Health Sciences Research Institute (ICVS), University of Minho, Braga, Portugal, [4]Pathology (LIM-14), University of Sao Paulo School of Medicine, Sao Paulo, Brazil

Corresponding author's email: claudia.oliveira220@fm.usp.br

Aims: To assess pathological and clinical aspects of patients with HCC secondary to NASH as related to immunohistochemical markers of glycolytic metabolic phenotype.

Material and Methods: 35 HCC specimens from 21 patients diagnosed with NAFLD undergoing liver resection (13 patients) or liver transplantation (9 patients) or both (1 patient) from 2005 to 2015. Demographic, clinical and biochemical data were related to histological features and immunohistochemical reactivity for K19, Ki-67, monocarboxylate transporter (MCT) 4 and 1, glucose transporter-1 (GLUT1) and carbonic anhydrase IX (CAIX).

Results: A total of 35 nodules were detected from 21 patients. Cirrhosis was present in 12 cases (7 F4A x 4F4B x 1F4C according to Laennec Staging) and 9 patients did not have cirrhosis (NASH staging: F2: 6pts, F3=3pts). Ages ranged from 50 to 77 years and 16 patients were male (76%). Sixteen patients (76%) had diabetes mellitus, 17 patients (81%) had arterial hypertension and 19 patients (90%) had BMI above 25kg/m2. HCC occurred in 8 patients Child A, 4 Child B and in 9 patients without cirrhosis. Alpha-fetoprotein level was normal in 13 patients. The mean survival time was 38.1 months. According to criteria defined by Salomão *et al*, 2011, 25 (70%) of nodules were diagnosed as "steatohepatitic HCC". Although 63% were poorly differentiated (G.3/ G.4) according to Edmondson & Steiner (1954), only 30% presented high levels of Ki 67 (>10%) and were positive for K19 (> 5%), which was associated to higher intratumoral inflammation (G 2/3) Interestingly, 75% of the patients with high Ki67 expression (>10%) were non-cirrhotic. MCT4 and GLUT1 expression was higher in nodules with more intratumoral fibrosis (G.3/G.4) and in nodules with more advanced BCLC B or C stages. GLUT1 expression was related to higher steatosis, higher parenchymal inflammation and marked balloonization.

Conclusions: 1. NASH-related HCC was found in both cirrhotic or non-cirrotic patients metabolic syndrome.sometimes with normal level of alpha-fetoprotein. **2.** Histological markers of "steatohepatitic HCC" were highly prevalent and the expression of glycolytic metabolic phenotype markers were higher in HCC with BCLC B or C. The expression of GLUT1 correlated with features associated with poor prognosis.

Disclosure of Interest: None Declared

P03-02YI

Enhanced urinary excretion of Thromboxane B2 in non-alcoholic fatty liver disease. Implication for antiplatelet treatment

Francesco Baratta[*1,2], Maria Del Ben[2], Daniele Pastori[1,2], Giancarlo Labbadia[2], Marta Novo[2], Roberto Carnevale[2], Francesco Angelico[3], Francesco Violi[2]

[1]Anatomical, Histological, Forensic Medicine and Orthopedic Science, [2]Internal Medicine and Medical Specialties, [3]Public Healt and Infectious Diseases, Spaienza- Università di Roma, Roma, Italy

Corresponding author's email: francesco.baratta@uniroma1.it

Introduction: Non-alcoholic fatty liver disease (NAFLD) represents the most common and emerging liver disease worldwide. Patients with NAFLD have an increased chance of developing cardiovascular diseases, such as myocardial infarction and stroke, which represent the major causes of death in this setting. Platelets play a key role in precipitating cardiovascular events as indicated by interventional studies with aspirin, which significantly reduces cardiovascular events in patients at risk or with acute cardiovascular events by impairing platelet thromboxaneB2 biosynthesis (TxB2) via irreversible acetylation of COX1.

Aims: Aim of the present study was to investigate the behaviour of platelet activation in patients with NAFLD and NASH and its impact with the degree of liver damage.

Material and Methods: We enrolled 44 consecutive patients with biopsy proven NASH and two other groups, matched for age, gender and BMI: 50 patients with simple steatosis (SS) diagnosed by ultrasound and with normal serum liver enzymes, and 50 control subjects without hepatic steatosis. Urinary TxB2 and serum bacterial lipopolysaccharide (LPS) were measured with ELISA test.

Results: Groups were well balanced for factors affecting platelets function (anti-platelet drugs, statin use, smoking status, diabetes and arterial hypertension). NASH patients, as compared to SS and controls, had increased median urinary TxB2 (165.0 vs 150.0 vs 55.5 ng/mg of creatinine, respectively [p<0.001]) and increased median serum LPS (109.2 vs 77.0 vs 49.5 pg/ml, respectively [p<0.001]). At bivariate analysis TxB2 was positively correlated with LPS (rs=0.608, p<0.001), GGT (rs=0.219, p0.010), AST (rs=0.181, p=0.030) and ALT (rs=0.192, p=0.021). At logistic regression, age (OR=1.074, p=0.006), LPS above median (OR=10.205, p<0.001) and liver diagnosis (controls vs SS: OR=5.881, p=0.001 and controls vs NASH: OR 12.390, p<0.001) were independently associated with TxB2 above median.

Conclusions: Patients with fatty liver disease display enhanced urinary excretion of TxB2 suggesting the existence of in vivo platelet activation in this setting. The positive correlation between urinary TxB2 and markers of liver damage such as GGT, ALT and AST suggests a relationship between liver damage and platelet activation. We found an increased LPS according to liver damage and a significantly association between circulating LPS and TxB2, suggesting the hypothesis of a role of this endotoxin as a stimulus for platelet activation.

Disclosure of Interest: None Declared

P03-03

Obeticholic acid differentially alters nash and plasma lipids in preclinical nash models

Francois Briand[*] [1], Emmanuel Brousseau[2], Isabelle Urbain[1], Marjolaine Quinsat[1], Thierry Sulpice[1]

[1]physiogenex, Labege, France, [2]physiogenex, Physiogenex, Labege, France

Corresponding author's email: f.briand@physiogenex.com

Introduction: Activation of farnesoid X receptor (FXR) represents a major therapeutic target to treat non-alcoholic steato-hepatitis (NASH). Obeticholic acid (OCA), a synthetic FXR agonist evaluated in clinical trials, is a benchmark in preclinical models.

Aims: We aimed to evaluate and differentiate OCA on key physiological features of NASH and dyslipidemia in several preclinical NASH models.

Material and Methods: Effects of OCA were evaluated in methionine choline deficient (MCD) diet-fed mice, Diet-Induced NASH (DIN) obese and insulin resistant C57BL6/J and LDL-receptor knock-out (LDL-r KO) mice fed a high fat/cholesterol/fructose rich diet, or DIN obese and insulin resistant hamster model fed a cafeteria diet. In each NASH model, plasma lipids were measured, liver histology and NAS scores quantified at the end of treatment.

Results: Compared to vehicle, OCA significantly reduced total plasma cholesterol levels by 64% in the MCD mice. In the liver, total cholesterol levels were increased by 31% and triglycerides were decreased by 24% by OCA (both $p<0.01$). Significant reduction in hepatic steatosis and ballooning scores were observed with OCA, but not for inflammation and fibrosis scores.
In the DIN C57BL6/J mouse model, OCA significantly reduced total plasma cholesterol levels and LDL-cholesterol levels by 33% and 45%, respectively. Significant reduction in hepatic steatosis, inflammation, hepatocyte ballooning, and fibrosis scores were observed with OCA. In the DIN LDL-r KO, OCA also reduced plasma total cholesterol by 56% and liver lipids significantly, but only reduced hepatocyte ballooning score. Additionally, OCA significantly reduced atherosclerotic plaque area by 25%, as well as inflammation and lipid accumulation.
In the DIN hamster model, OCA significantly reduced hepatic inflammation score and tended to reduce hepatic steatosis and ballooning. Due to its closer lipoprotein metabolism to humans, hamsters treated with OCA showed significantly higher LDL-cholesterol levels (+27%) and lower HDL-cholesterol levels (-20%).

Conclusions: According to the preclinical model, OCA alters plasma lipids and NASH differentially. While DIN C57BL6/J mice replicates the benefits on NASH, the DIN hamster is the only model showing the same dyslipidemic side effect observed in humans. These models' differences should be considered when evaluating new drug compounds compared with OCA.

Disclosure of Interest: F. Briand: Employee: Physiogenex, E. Brousseau: Employee: Physiogenex, I. Urbain: Employee: Physiogenex, M. Quinsat: Employee: Physiogenex, T. Sulpice: Employee: Physiogenex

P03-04YI

A retrospective study looking at patients diagnosed with an incidental hepatocellular carcinoma and their shared characteristics

Frazer Warricker*[1], Janisha Patel[1]

[1]*Department of Hepatology, University Hospital Southampton, Southampton, United Kingdom*

Corresponding author's email: f.warricker@gmail.com

Introduction: Mortality and morbidity related to primary liver cancer has risen steadily for forty years. Hepatocellular carcinoma (HCC) constitutes 70-90% of all primary liver cancers, is the sixth commonest cancer and third largest cause for cancer related mortality worldwide. Metabolic syndrome (MetS) is a major global public health concern and is a combination of physiological, biochemical, clinical and metabolic factors which contribute to an increased risk of cardiovascular disease, type 2 diabetes mellitus (T2DM) and all-cause mortality. Obesity and T2DM are strongly associated with developing non-alcoholic steatohepatitis (NASH), with the incidence of HCC in NASH cirrhosis being 2.6% per year.

Aims: To highlight factors associated with incidental findings of HCC at the University Hospital Southampton (UHS) focussing on characteristics consistent with MetS.

Material and Methods: A retrospective review of electronic medical records in patients with a diagnosis of HCC made at UHS between 2012 and 2016, either found incidentally or through routine surveillance. Electronic medical records for each patient were used to look at characteristics including: BMI, age, gender, co-morbidities consistent with MetS and duration of deranged liver function tests.

Results: From 2012 to 2016 the annual incidence of HCC's found through surveillance remained relatively consistent with an average of 12 patients per year (60 in total). However when looking at incidental HCC's found over the same period, the incidence steadily increased from 16 patients in 2012 to 27 in 2016.
In patients with a diagnosis of incidental HCC, 56.9% had a BMI of greater than 25 (11 out of 102 (10.7%) cases had no recorded BMI), 66.6% had co-morbidities consistent with metabolic syndrome with 67.6% below 75 years old when diagnosed with an HCC. In addition, 71.6% diagnosed with HCC were male and 28.4% female. Patients with incidental HCC had mildly elevated liver functions tests (less than three times the upper limit of normal) detected in primary care with a median duration of greater than 6 years prior to diagnosis.

Conclusions: The incidence of incidental HCC's has risen from 2012 to 2016 and is associated with the following factors: Male sex, < 75 years old, BMI over 25, co-morbidities consistent with metabolic syndrome and advanced liver disease or cirrhosis. Identifying patients earlier in their disease pathway in primary care, could improve access to HCC surveillance in secondary care, resulting in earlier diagnosis and wider access to treatment.

Disclosure of Interest: None Declared

P03-05

Application of a non-invasive integrated screening approach to facilitate enrollment of clinical trials with investigational NAFLD/NASH therapeutics

Gerardo Rodriguez-Araujo[*] [1], Moises Hernandez[1], Christian Weyer[1], Linda Morrow[1], Laura Millán[2], Ibon Martínez-Arranz[2], Marcus Hompesch[1]

[1]ProSciento, Inc., Chula Vista, United States, [2]OWL Metabolomics, Derio, Spain

Corresponding author's email: gerardo.rodriguez@prosciento.com

Introduction: Despite NAFLD/NASH being a prevalent co-morbidity in obesity and type 2 diabetes, clinical development of NAFLD/NASH therapeutics is currently hindered by significant challenges in clinical trial enrollment. Traditional study screening is commonly associated with screen failure rates of up to 80%. Given the cost and timeline implications of subjects failing to meet MRI- or biopsy-based inclusion criteria, there is a clear need for optimized screening strategies in NAFLD/NASH clinical studies.

Aims: Our goal was to assess the potential utility of an integrated screening approach aimed at pre-identifying individuals who have a high probability of being eligible for NAFLD/NASH clinical studies.

Material and Methods: Our integrated screening approach consists of: 1) a scoring system based on specific equation matrixes from 2 validated published NAFLD screening algorithms; and 2) a validated lipidomic testing panel for NAFLD and NASH, developed by One Way Liver (OWL) Metabolomics. OWL assays include profiling of 28 triglycerides. Testing validated against liver biopsy. We applied this scoring system (score of 0 to 5, with 5 having the highest probability of NAFLD) to two cohorts of subjects (Cohort 1: 204 obese type 2 diabetic subjects; Cohort 2: 55 obese nondiabetic subjects). Cohort 1 subjects with a score of 5 underwent MRI-PDFF-based quantitation of liver fat (%), whereas cohort 2 subjects with a score of 5 underwent additional lipidomic testing to further characterize the probability of NAFLD and NASH.

Results: Of the 18 subjects from cohort 1 with a score of 5, 15 (83%) had >10% liver fat by MRI-PDFF (range: 12.5 - 27.8%). Algorithm's detection profiling of >10% liver fat had a Se of 88.24% (95%CI 0.63-0.98), Sp of 62.50% (95%CI 0.24-0.91), PPV of 83.33% (95%CI 0.66-0.92) and NPV of 71.43% (95%CI 0.37-0.91). Of the 13 subjects from cohort 2 with a score of 5, all (100%) tested positive for NAFLD lipidomic index and of those, 5 (38%) also tested positive for NASH lipidomic index. MRI-PDFF data will be presented at the meeting.

Conclusions: These preliminary results point to the potential utility of optimized, non-invasive screening algorithms for NAFLD/NASH studies. Pre-screening strategies to identify individuals who are most likely to have significant steatosis or steatohepatitis on MRI or biopsy, may be a scalable, efficient means of reducing screen failure rates in NAFLD/NASH clinical trials. Refinement and validation in larger cohorts is currently underway.

Disclosure of Interest: G. Rodriguez-Araujo: Employee: ProSciento, Inc, M. Hernandez: Employee: ProSciento, Inc, C. Weyer: Employee: ProSciento, Inc, L. Morrow: Employee: ProSciento, Inc, L. Millán: Employee: OWL Metabolomics, I. Martínez-Arranz: Employee: OWL Metabolomics, M. Hompesch: Employee: ProSciento, Inc

P03-06

How many HIV mono-infected or HBV or HCV co-infected patients with undetectable viremia should be monitored for liver disease severity in the presence of suspected NAFLD?

Giovanni Guaraldi[*] [1], Iacopo Franconi[1], Marianna Menozzi[1], Andrea Malagoli[1], Antonella Santoro[1], Vanni Borghi[2], Giulia Besutti[1], Filippo Schepis[1], Amedeo Lonardo[2], Cristina Mussini[1]

[1]University of Modena and Reggio Emilia, [2]Azienda Ospedaliero-Universitaria di Modena, Modena, Italy

Corresponding author's email: giulia.besutti@libero.it

Introduction: HIV infection is associated with fatty liver as a result of host factors, HIV per se, or (HCV or HBV) co-infections, the so called "virus-associated fatty liver disease" (VAFLD) even in the absence of detectable HIV, HBV or HCV viremia.
EASL have recently issued a diagnostic flow-chart, to assess and monitor NAFLD disease in virus-free patients based on fatty liver screening and assessment of transaminase (GPT) and non-invasive fibrosis score (Fib-4).

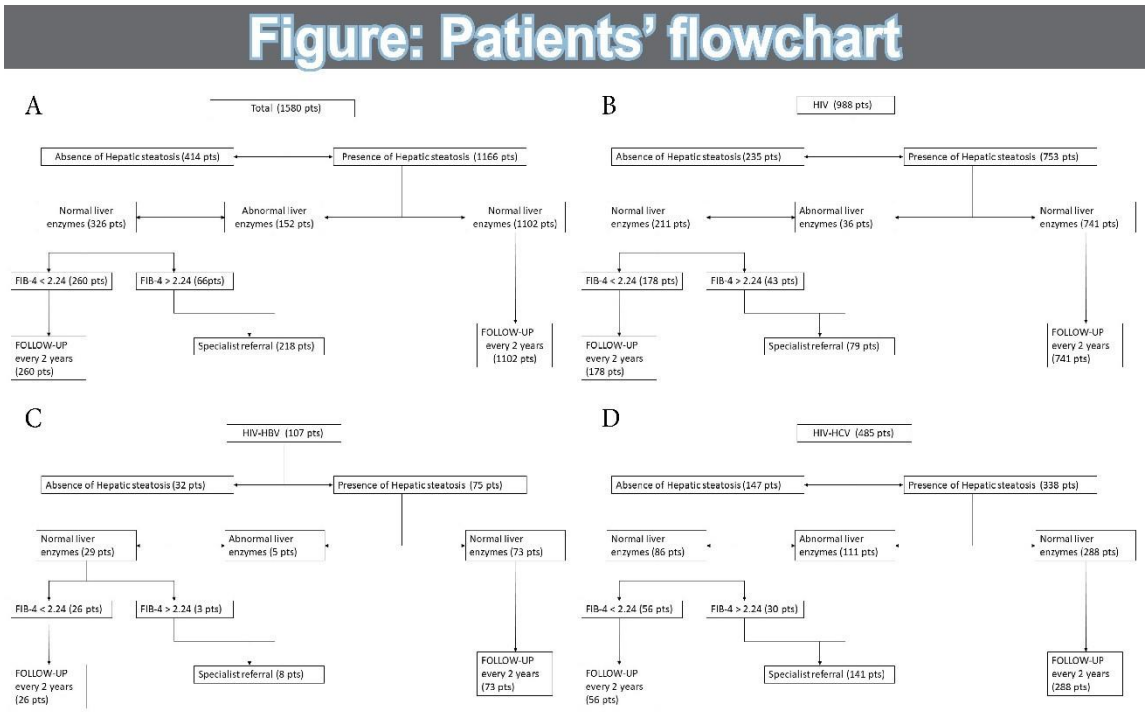
Aims: The objective of this study was to estimate and characterize HIV patients at risk for VAFLD using EASL- Clinical Practice Guidelines.

Material and Methods: This cross-sectional study was conducted on patients attending the Modena HIV Metabolic Clinic.
Inclusion criteria were: patients with no high alcohol introduction (<2 alcohol unit/day) and HIV mono-infected or HBV/HIV or HCV/HIV co-infected (all with undetectable viremia). Exclusion criteria were any HIV-RNA, HBV-DNA or HCV-RNA detectability.
Fatty liver screening was offered per standard of care using abdominal CT (radiological criteria Liver/Spleen<1.1). Liver enzymes (GPT) cut-off for women was 19 IU, for men was 30 IU and Fib-4 cut-off was >2.64.

Results: The whole population included 1580 patients (50 ±8 years, 28% females). Of them, 988 (62.5%) were HIV-monoinfected, 107 (6.8%) were HBV/HIV coinfected, and 485 (30.7%) were HCV/HIV coinfected.
Figure 1 depicts EASL flowchart applied to all included patients (A), HIV monoinfected patients (B), HBV/HIV patients (C), and HCV/HIV patients (D). 13,8% of this HIV cohort (including 7% of HIV mono infected, 7.5% of HBV/HIV and 29% of HCV/HIV patients) are at risk of progressive liver disease and should be referred to hepatology consultation.
At logistic regression analyses, independent predictors for VAFLD were: male gender (OR=0.62, CI95%:0.46-0.83), duration of HIV infection per month (OR=1.01, CI95%:1.01-1.01, p<0.01), HOMA-IR (OR=1.13, CI95%1.07-1.2, p<0.01), and sHIV-cHCV group (OR=2.16 CI95%: 1.60-2.92 p<0.01), after correction for age, gender, CD4 nadir, current CD4, PCR, D-Dimer.

Conclusions: This study suggests the need for dedicated diagnostic hepatological monitoring of HIV mono and co-infected patients with undetectable viremia.

Figure:



Disclosure of Interest: None Declared

P03-07YI

Liver steatosis quantification in HIV-infected patients: comparison between Magnetic Resonance Imaging (MRI) techniques and Magnetic Resonance 1H Spectroscopy (MRS)

Giulia Besutti[*] [1], Guido Ligabue[1], Antonella Santoro[1], Luca Nocetti[2], Riccardo Scaglioni[2], Stefano Zona[1], Giovanni Guaraldi[1]

[1]University of Modena and Reggio Emilia, [2]Azienda Ospedaliero-Universitaria Policlinico di Modena, Modena, Italy

Corresponding author's email: giulia.besutti@libero.it

Introduction: HIV infection is associated with fatty liver as a result of multiple viral and host factors. The assessment of Non Alcoholic Fatty Liver Disease (NAFLD) is crucial for the evaluation of overall metabolic health among HIV-infected patients, therefore non-invasive accurate biomarkers of liver steatosis are needed.

Aims: The purpose of this study was to evaluate different MRI techniques in the quantitative assessment of liver steatosis, using MRS as the reference standard.

Material and Methods: Forty-five HIV-infected patients (52 ± 9 years, 15.56% females) underwent MR examination for steatosis assessment. Liver Fat Content (LFC) was estimated by means of MRS and two MRI techniques: Dual-Phase T1-weighted Gradient-Echo and Multiecho Gradient-Echo. For this last technique, LFC was calculated both on the same single voxel used for Spectroscopy (SV-Multi-LFC) and on the whole liver parenchyma with two different methods: selecting 12 elliptical Regions of Interest on three different slices (12ROI-Multi-LFC) and selecting three free-hand ROIs comprehending the whole liver volume on the same slices (WV-Multi-LFC). The associations between LFC calculated with different techniques were measured with univariate linear regression analysis after normalization of non-normally distributed variables. Wilcoxon matched-pairs signed-ranks test corrected for multiple comparison was used to evaluate differences among LFCs calculated with the different MR techniques. Areas under receiver operating characteristic (ROC) curves were used to compare different MRI methods, using a cut-off value of 5% MRS LFC.

Results: Strong associations were found between MRS LFC and Dual-Phase LFC (R^2=0.96; p<0.001, ß=1.23), SV-Multi-LFC (R^2=0.96; p<0.001, ß=0.95), 12ROI-Multi-LFC (R^2=0.94; p<0.001, ß=0.93), and WV-Multi-LFC (R^2=0.93; p<0.001, ß=0.96). Results of Wilcoxon matched-pairs signed-ranks test are reported in table. Significant differences were found among Dual-Phase LFC and MRS LFC as well as Multi-echo LFCs. Area under the ROC curves ranged from 0.99 to 1.00 for the four MRI methods, without any significant difference among them.

Conclusions: In this cohort of HIV-infected patients, MRI techniques resulted reliable in quantitative steatosis assessment when compared to MRS. LFC estimated with all imaging techniques was strongly associated with MRS LFC. Dual-Phase, the most used in clinical practice, was found to overestimate LFC by a 23% when compared to MRS, whereas other techniques showed more accurate results (≤7% discrepancy).

Figure:

P-values	MRS LFC	Dual-Phase LFC	SV-Multi-LFC	12ROI-Multi-LFC
Dual-Phase LFC	**<0.001**			
SV-Multi-LFC	0.14	**0.002**		
12ROI-Multi-LFC	0.10	**0.010**	0.82	
WV-Multi-LFC	**0.003**	0.07	0.05	**<0.001**

Disclosure of Interest: None Declared

P03-08YI

L-Selectin drives development and progression of non-alcoholic steatohepatitis (NASH) in mouse and man

Hannah K. Drescher[*] [1], Angela Schippers[2], Hacer Sahin[1], Christian Trautwein[1], Konrad L. Streetz[2], Daniela C. Kroy[1]

[1]Department of Internal Medicine Iii, [2] University Hospital Rwth Aachen, Aachen, Germany

Corresponding author's email: hdrescher@ukaachen.de

Introduction: Non-alcoholic steatohepatitis (NASH) is the third most common reason for liver transplantation and a growing medical problem.

Aims: The significance of infiltrating lymphocytes in NASH development remains unclear. This study investigates the role of the cell adhesion molecule L-Selectin (CD62L) in patients with steatosis and in two different mouse steatohepatitis models.

Material and Methods: Levels of soluble L-Selectin (sL-Selectin) were analysed in serum of patients with acute and chronic liver diseases. Hepatic L-selectin expression was measured in patients with different stages of steatosis and NASH. Furthermore, constitutive L-Selectin$^{-/-}$ mice were fed MCD-diet (methionine and choline deficient) for 4 weeks or HF-diet (high fat) for 24 weeks.

Results: Patients with NAFLD and acute liver injury display increased sL-Selectin serum levels. Interestingly serum levels also increase in patients after treating ulcerative colitis (UC) with Entyvio® but not in patients with UC per se. Hepatic expression of L-Selectin dramatically increases in NASH patients (Figure 1). Coherent with the human data, MCD (4 weeks) and HFD (24 weeks) treatment of L-Selectin$^{-/-}$ mice showed a less invasive phenotype in steatosis development compared to WT. This was reflected by maintenance of an intact liver architecture and less fatty liver degeneration. Furthermore, L-Selectin$^{-/-}$ animals displayed a dampened manifestation of the metabolic syndrome with decreased liver:body weight ratio, an improved insulin resistance and decreased cholesterol and triglyceride levels in both mouse models. µCT analysis strengthen these observations through visualisation of less total bodyfat accumulation in L-Selectin$^{-/-}$ mice. The amelioration of steatohepatitis was further reflected by lower transaminases and pro-inflammatory cytokines in L-Selectin$^{-/-}$ mice. Consistent with the less invasive phenotype, L-Selectin$^{-/-}$ animals showed an increased anti-oxidative stress response by elevated expression of Nrf2 and HO-1 and enhanced hepatic immune cell infiltration of T_{Reg} cells. Those changes finally resulted in a protection of L-Selectin$^{-/-}$ mice from fibrosis progression.

Conclusions: L-Selectin is increased in patients with fatty liver disease. In order to analyse the underlying mechanisms, we could show that L-Selectin deficiency in mice leads to a protection against diet induced steatohepatitis. Therefore, the blockade of L-Selectin provides a novel target for therapeutic interventions during NASH development.

Figure:

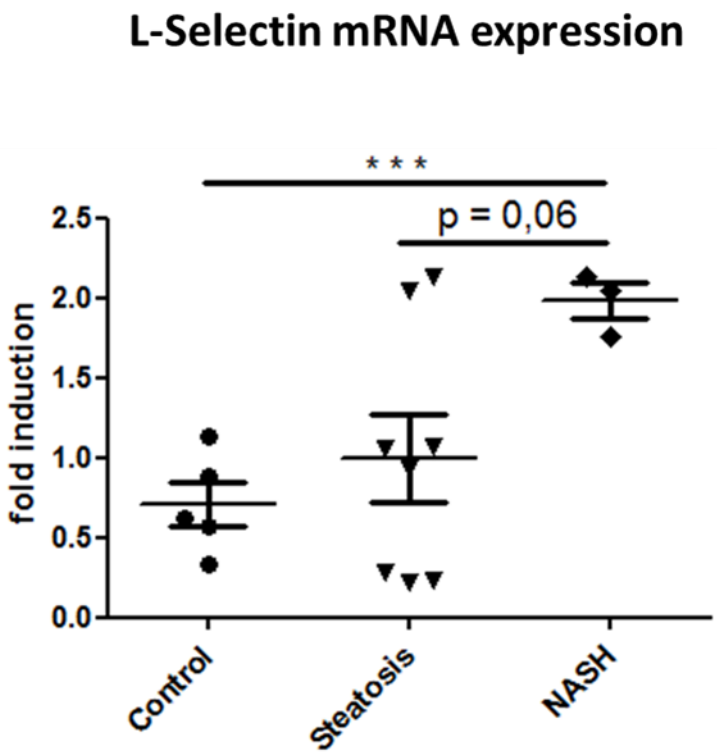


Figure 1 : Increased hepatic L-Selectin mRNA expression in NASH patients

L-Selectin mRNA expression: Whole liver extracts of control patients, patients with steatosis or NASH were analyzed for mRNA expression via Real-Time PCR. For quantification values are expressed as fold induction over the mean values obtained for control patients. All results are expressed as mean ± SEM, p values were measured by Student's t test. *** p<0,001.

Disclosure of Interest: None Declared

P03-09

Liver and circulating long non-coding RNA GAS5 is correlated with liver fibrosis in patients with non-alcoholic fatty liver disease

Jung Gil Park[*1], Soo Young Park[2], Keun Hur[3], Heon Ju Lee[1], Won Young Tak[2], Young Oh Kweon[2], Se Young Jang[2], Yu Rim Lee[2], Gyeonghwa Kim[3], Jihyun Lee[3], Eunhye Lee[3], Hye Won Lee[4]

[1]Internal medicine, Yeungnam University Hospital, [2]Internal medicine, [3]Biochemistry and Cell Biology, Kyungpook National University, [4]Pathology, Keimyung University, Daegu, Korea, South

Corresponding author's email: gsnrs@naver.com

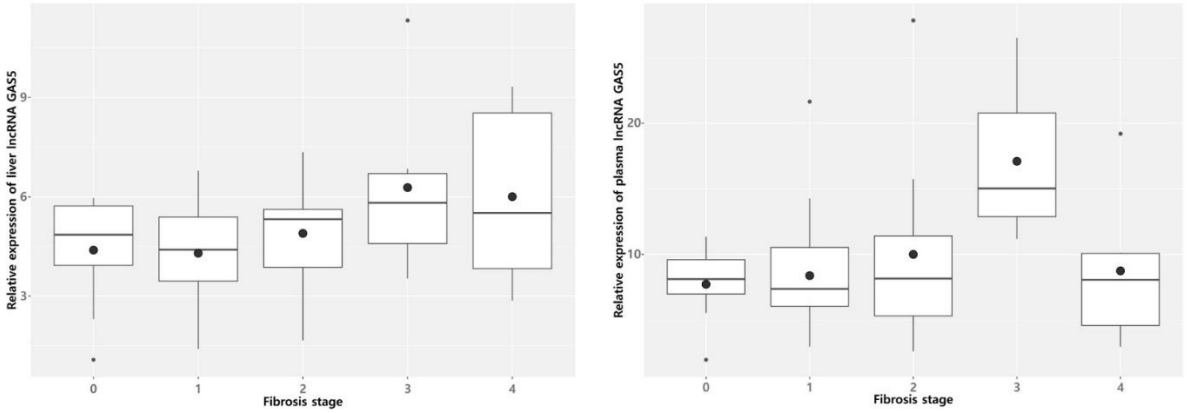
Introduction: Non-alcoholic steatohepatitis is an important process in progressing liver fibrosis. However, presence of advanced liver fibrosis is more important prognostic factor in patients with non-alcoholic fatty liver disease (NAFLD). Long non-coding RNA (lncRNA) GAS5 is related with inhibition of liver fibrogenesis by competing endogenous RNA.

Aims: To evaluate the both liver and circulating lncRNA GAS5 in patients with NAFLD.

Material and Methods: We analyzed a total of fifty-one patients with NAFLD confirmed by percutaneous liver biopsy, which is reviewed by one pathologist. Expression of lncRNA GAS5 in both liver and plasma was analyzed using quantitative real-time polymerase chain reaction in NAFLD patients according to stage of fibrosis (F0; n=8, F1; n=21, F2; n=10, F3; n=6, F4; n=6)

Results: Expression of lncRNA GAS5 was significantly increased in patients with advanced fibrosis compared to patients without advanced fibrosis (liver, 4.5±1.6 vs 6.3±2.8, *p*=0.026; plasma, 8.7±4.9, 17.1±6.0, *p*=0.000). As the fibrosis was progressed, expression of lncRNA GAS5 in both liver and plasma was increased except cirrhosis (liver; *r*-value=0.305; *p*=0.042, plasma; *r*-value=0.452; *p*=0.002). However, expression of lncRNA GAS5 in plasma was decreased in patients with cirrhosis compare to advanced fibrosis (8.8±5.9 vs 17.1±6.0, *p*=0.036).
Conclusions: Both liver and circulating LncRNA GAS5 is correlated with stage of liver fibrosis before cirrhosis occurs.

Figure:



Disclosure of Interest: None Declared

P03-10

Method of assessing the functional reserve of the liver-13C-methacetin breath test

Igor Bakulin*[1], Natalia Bakulina[1], Olga Medvedeva[1], Liya N. Belousova[1], Tatiana Ilchishina[2], Yury Medvedev[1]

[1]NWSMU named after I.I.Mechnikov, [2]The Federal State Budgetary Institute «The Nikiforov Russian Center of Emergency and Radiation Medicine» The Ministry of Russian Federation for Civil Defense, Emergencies and Elimination of Consequences of Natural Disasters., Saint Petersburg, Russia

Corresponding author's email: liya73@mail.ru

Aims: determination of the functional reserve of the liver depending on the stage of fibrosis in patients with chronic liver diseases of various etiologies.

Material and Methods: The study included 40 patients with chronic liver diseases of different etiology including NAFLD. The stage of liver fibrosis was determined according to the current diagnostic standards (fibrotests and / or elastometry, liver biopsy). Patients were divided into 3 groups: 1 (n=16) with fibrosis F0; 2 (n=13) F1-2; 3 (n=11) F3-4. Functional liver reserve was estimated by a 13C-metacetin breath test in all patients. The indices are: cumulative dose of 13C for 60 minutes (CUM-60, for healthy individuals this figure is 20.4 ± 2.6%), cumulative dose for 120 minutes (CUM-120, in healthy persons this figure is 25.9 - 38.7%), dose in hour on the 20th minute of the test (Dose/h-20, in healthy individuals this figure is 28.6%), delta over the baseline in the 20th minute (DOB-20, in healthy individuals this figure is 22.06 ± 2.4). The CUM-60 and CUM-120 measures the functional liver reserve, and the Dose/h-20 and DOB-20 indicators show the metabolic rate in the CYP450 system.

Results: Analysis of data obtained during the ^{13}C-MBT showed significant differences between the groups for all the indicators being evaluated. The functional liver reserve was reduced in all three groups, with the greatest deviations from normal values observed in patients with F3-4. CUM-60 in the 1st group was 16.38 ± 1.08%, in the 2nd group - 15.04 ± 1.31%, in the third group - 10.75 ± 1.44%, respectively. CUM-120 in the 1st group was 25.06 ± 1.5%, in the 2nd and 3rd groups - 23.04 ± 1.86% and 17.95 ± 2.16%, respectively. Dose/h-20 in 1 - 24.51 ± 2.02%, in the 2nd and 3rd groups - 21.04 ± 2.52% and 14.23 ± 2.85%, respectively. DOB-20 in group 1 - 17.86 ± 1.47 ‰, in groups 2 and 3 - 14.22 ± 1.55 ‰ and 9.45 ± 1.98 ‰, respectively. For all indices, the differences between groups 1 and 3 were statistically significant (p≤0.01).

Conclusions: In patients with chronic liver diseases a decrease in the functional reserve of the liver is detected. At the same time, the degree of decrease in the functional reserve correlates with the stage of liver fibrosis. The CUM-60 and CUM-120 show the greatest diagnostic significance in assessing the functional reserve of the liver.

Disclosure of Interest: None Declared

P04-01

Mir-181 as a noninvasive biomarker to identify advanced fibrosis in NASH patients

Fernanda Malta[1], José Tadeu Stefano[1], Rodrigo V. Lima[1], Ana Paula M. Salles[1], Venancio A. F. Alves[2], Flair José Carrilho[1], João Renato R. Pinho[1], Claudia P. Oliveira[*1]

[1]gastroenterology - Lim-07, [2]pathology - Lim-14, University of Sao Paulo School of Medicine, Sao Paulo, Brazil

Corresponding author's email: claudia.oliveira220@fm.usp.br

Introduction: Circulating microRNAs (miRNAs) have emerged as attractive candidate of noninvasive biomarkers for early detection and monitoring Nonalcoholic fatty liver disease (NAFLD progression).

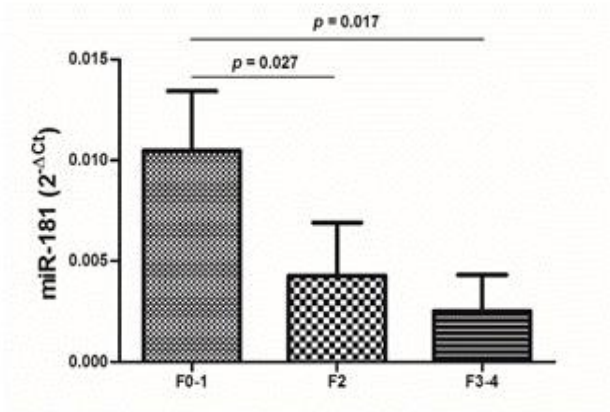
Aims: To evaluate the potencial of five selected serum microRNAs (miR-21, miR-29, miR-122, miR-155 and miR-181a) as non-invasive biomarkers to stage liver fibrosis in nonalcoholic steatohepatites (NASH).

Material and Methods: Forty five well characterized patients with biopsy proven NASH were studied. All biopsies were scored using the NASH CRN Score by an expert pathologist. NASH was defined by the presence of each component of the NAFLD Activity Score (NAS). MicroRNAs expression was assayed by qPCR. Clinical data of three independent fibrosis group (F0-1, F2 and F3-4) were compared using one-way ANOVA followed by Dunn's multiple comparisons test. The miRNA expression levels (2-dCT) were compared using the nonparametric Mann-Whitney U test. Correlations between parameters were determined by Spearman correlation. P<.05 was considered significant, with 95% confidence interval.

Results: Advanced fibrosis was seen in older patients than younger (64 (58.5 - 70.2) vs 57.5 (50.5 - 60.2; p=0.02). Serum miR-181a expression was decreased in patients with advanced fibrosis (F3-F4; p= 0.017) as well F2 (p= 0.027) compared with early stages of fibrosis (F0-F1) in NASH patients (Figure 1). Besides, we observed a slightly negative correlation (r= -0.332; p= 0.038) between AST and miR-181a, suggesting that when exist advanced fibrosis, AST increase and miR-181a expression decrease. In the other hand, miR-21, miR-29, miR-122, miR-155 did not identify advance stages of fibrosis in NASH patients, however, according metabolic syndrome features, the presence of arterial hypertension was correlated with higher miR-21 and 29 (p=0.005; p=0.007).

Conclusions: miR-181a can be a new nonivasive biomarker for advanced fibrosis in NASH patients and miR-21, miR-29 identified NASH patients with arterial hypertension. Further studies are needed to confirm these data in a larger cohort.

Figure:



Disclosure of Interest: None Declared

P04-02YI

Non-alcoholic fatty liver disease and adherence to Mediterranean diet

Maria Del Ben[1], Francesco Baratta[* 2], Daniele Pastori[2], Licia Polimeni[1], Tommaso Bucci[3], Fabrizio Ceci[4], Ilaria Ernesti[5], Cinzia Calabrese[1], Francesco Violi[1], Francesco Angelico[6]

[1]Internal Medicine and Medical Specialities, [2]Anatomical, Histological, Forensic Medicine and Orthopedics Sciences, Sapienza University, Rome, [3]Internal Medicine and Medical Specialities, University of Salerno, Salerno, [4]Cellular Biotechnologies and Hematology, [5]Experimental Medicine, Section of Medical Pathophysiology, Endocrinology and Nutrition, [6]Public Health and Infectious Diseases, Sapienza University, Rome, Italy

Corresponding author's email: francesco.angelico@uniroma1.it

Introduction: The prevalence of cardio-metabolic disorders, including non-alcoholic fatty liver disease (NAFLD), is increasing in western countries, due to changes in lifestyle and dietary habits. Mediterranean Diet (Med-Diet) is effective for cardiovascular prevention, but its relationship with NAFLD has been scarcely investigated.

Aims: To assess the relationship between adherence to Mediterranean Diet and prevalence of NAFLD in a large sample of adult outpatients with cardiometabolic risk factors.

Material and Methods: We included 584 consecutive outpatients presenting with ≥1 cardiovascular risk factor such as type 2 diabetes mellitus (T2DM), arterial hypertension, overweight/obesity and dyslipidaemia. Liver steatosis was assessed by ultrasonography. Med-Diet adherence was investigated by a validated semi-quantitative 9-item dietary questionnaire; patients were divided into low, intermediate and high adherence. Insulin resistance was defined by the 75th percentile of HOMA-IR (≥3.8).

Results: Mean age was 56.2±12.4 years and 38.2% were women. Liver steatosis was present in 82.7%, and its prevalence decreased from low to high adherence group (96.5% vs. 71.4%, p<0.001). In a multiple logistic regression analysis, hypertriglyceridemia (Odds Ratio (OR):2.913; p=0.002), log(ALT) (OR:6.186; p<0.001), Med-Diet adherence (intermediate vs. low OR:0.115; p=0.041, high vs. low OR:0.093; p=0.030), T2DM (OR:3.940; p=0.003) and high waist circumference (OR:3.012; p<0.001) were associated with NAFLD. Among single foods, low meat intake (OR:0.178; p<0.001) was inversely significantly associated with NAFLD. In 334 non-diabetic NAFLD patients, age (OR:1.035, p=0.025), high waist circumference (OR:7.855, p<0.001), hypertriglyceridemia (OR:2.152, p=0.011) and Log(ALT) (OR:2.549, p=0.002) were directly associated with HOMA-IR, while Med-Diet score was inversely associated (OR:0.801, p=0.018).

Conclusions: We found an inverse relationship between Med-Diet and NAFLD prevalence. Among NAFLD patients, good adherence to Med-Diet was associated with lower insulin resistance.The association of poor adherence to Med-Diet with a higher prevalence of NAFLD and insulin resistance suggest that nutritional habits should be always investigated in patients with cardiometabolic risk factors. Our findings suggest that Med-Diet may be a beneficial nutritional approach in NAFLD patients.

Disclosure of Interest: None Declared

P04-03YI

Effects of dark chocolate on endothelial function in patients with non-alcoholic steatohepatitis

Francesco Angelico[*] [1], Lorenzo Loffredo[2], Ludovica Perri[2], Simona Battaglia[2], Roberto Carnevale[2], Cristina Nocella[2], Marta Novo[2], Fabrizio Ceci[3], Francesco Violi[2], Maria Del Ben[2]

[1]Public Health and Infectious Diseases, [2]Internal Medicine and Medical Specialities, [3]Cellular Biotechnologies and Hematology, Sapienza University of Rome, rome, Italy

Corresponding author's email: francesco.angelico@uniroma1.it

Introduction: Oxidative stress plays a pivotal role to determine endothelial dysfunction in patients with non-alcoholic fatty liver disease. Polyphenols could reduce oxidative stress and improve endothelial function by inhibiting the nicotinamide-adenine-dinucleotide-phosphate (NADPH) oxidase isoform Nox2.

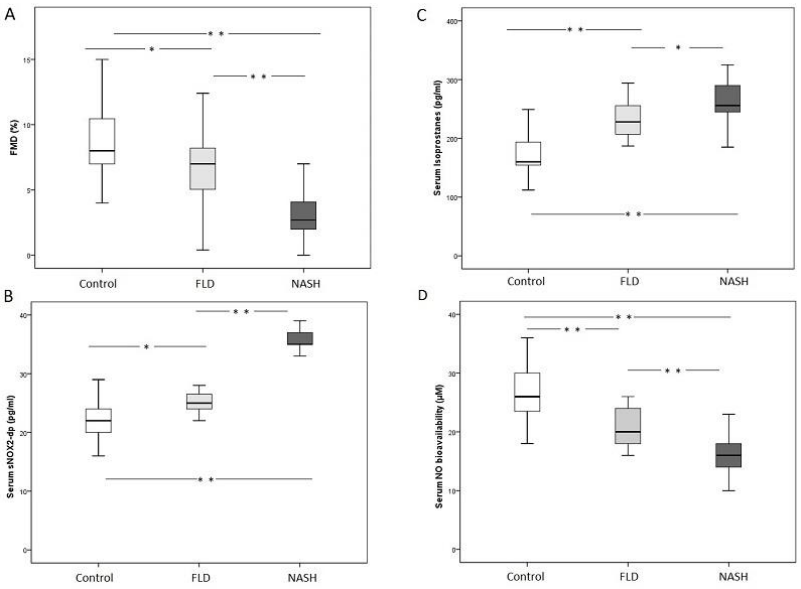
Aims: The aim of this study was to analyze the effect of cocoa polyphenols on endothelial function, assessed by flow-mediated dilation (FMD), in a population affected by non-alcoholic steatohepatitis (NASH).

Material and Methods: In a cross-sectional study we analyzed FMD and oxidative stress, as assessed by Nox2 activation, serum isoprostanes and nitric oxide bioavailability (NOx), in patients with NASH (n=19), simple fatty liver disease (FLD) (n=19) and controls (n=19). Then, we performed a randomized, cross-over study in 19 subjects with NASH comparing the effect of 14-days administration of 40 g of chocolate at high (dark chocolate, cocoa>85%) versus low content (milk chocolate, cocoa <35%) of polyphenols on FMD and oxidative stress.

Results: Compared to controls, NASH and FLD patients had higher Nox2 activity and isoprostanes levels and lower FMD and NOx, with a significant gradient between FLD and NASH. The interventional study showed that, compared to baseline, FMD and NOx increased (from 2.9±2.4 to 7.2±3.0%; p<0.001 and from 15.9±3.6 to 20.6±4.9 µM, p<0.001, respectively) in subjects given dark but not in those given milk chocolate. A simple linear regression analysis showed that Δ (expressed by difference of values between before and after 14 days of chocolate assumption) of FMD was associated with Δ of Nox2 activity (Rs=-0.323; p=0.04), serum isoprostanes (Rs:-0.553; p<0.001) and NOx (Rs:0.557;p<0.001).

Conclusions: Cocoa polyphenols improve endothelial function via Nox 2 down-regulation in NASH patients.

Figure:



Disclosure of Interest: None Declared

P04-04YI

Increased circulating proneurotensin levels identify non-alcoholic fatty liver disease in obese subjects with and without type 2 diabetes

Ilaria Barchetta[*] [1], Flavia Agata Cimini[1], Frida Leonetti[1], Danila Capoccia[1], Claudio Di Cristofano[1], Gianfranco Silecchia[1], Marju Orho-Melander[2], Olle Melander[2], Maria Gisella Cavallo[1]

[1]Sapienza University, Rome, Italy, [2]Lund University, Malmo, Sweden

Corresponding author's email: ilariabarchetta@hotmail.com

Introduction: Neurotensin (NT) is a peptide produced by specialized enteroendocrine cells of the small intestine and released in response to fat ingestion, facilitating fatty acids translocation in rat intestine. NT-deficient mice are protected by high fat diet-induced weight gain and hepatic steatosis; in humans, higher circulating NT are associated to obesity, increased risk of diabetes, cardiovascular diseases and cancer. No study in humans has investigated if NT is related to non-alcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH).

Aims: Therefore, the aims of this study were to investigate the relationship between plasma proneurotensin1-117 (pro-NT) levels, a stable fragment of the NT precursor hormone, and the presence and severity of biopsy-proven NAFLD/NASH and to unravel clinical correlates of increased pro-NT levels.

Material and Methods: For this cross-sectional study, we recruited 60 obese individuals undergoing bariatric surgery for clinical purposes. The study population underwent clinical and metabolic characterization; liver biopsies were performed during surgery. Plasma pro-NT levels were assessed using a chemiluminometric sandwich immunoassay.

Results: Thirty-two subjects (53%) had a histological diagnosis of NAFLD and showed significantly higher plasma pro-NT than those without NAFLD (183.6±81.4 vs 86.7±56.8 pmol/L, $p<0.001$). Greater pro-NT levels correlated with NAFLD presence ($p<0.001$) and severity ($p<0.001$), age, female gender, insulin-resistance and T2D. Higher pro-NT predicted NAFLD with AUROC=0.836 (C.I.95%:0.73-0.94, $p<0.001$; sensitivity: 84%, specificity: 75% at pro-NT> 107 pmol/L). Belonging to the highest pro-NT quartile was associated with increased risk of NAFLD (OR: 2.62; 95% CI 1.08-6.40), after adjustment for confounders.

Conclusions: This study demonstrates for the first time that increased plasma pro-NT levels identify the presence/severity of biopsy-proven NAFLD in obese subjects with and without T2D; indeed, in dysmetabolic individuals, NT may specifically promote hepatic fat accumulation though mechanisms likely related to increased insulin-resistance.

Disclosure of Interest: None Declared

P04-05

Discovery of selective and specific Collagen I translation inhibitors as antifibrotics, using fluorescent-labeled Glycine- and Proline tRNA

Iris Alroy*[1], Wissam Mansour[1], Dave Sheppard[1], Zeev Smilansky[1], Inbal Shapira Lots[1]

[1]*Anima Biotech, Ltd., Tel Aviv, Israel*

Corresponding author's email: iris.alroy@animabiotech.com

Introduction: Fibrotic diseases are characterized by overproduction of extracellular matrix (ECM) by activated fibroblasts, including a large component of collagen type 1 (**COL1**). Studies showed that collagen I production is regulated at both transcription and translation. We have developed a proprietary protein synthesis monitoring technology, by monitoring ribosome activity is live cells, into a high throughput assay, towards the discovery of COL1 translation modulators and development of anti-fibrosis drugs.

Aims: Setup of a high content screen monitoring COL1 translation in human lung fibroblasts by using a specific pair of isoacceptor tRNApro and tRNAGly and screen a diverse library of compounds towards the identification of compounds which specifically inhibit COL1A translation.

Material and Methods: Protein Synthesis Monitoring (**PSM**) is a novel technology that monitors the process of protein synthesis in living cells. In PSM, tRNAs labelled as FRET donors and acceptors are introduced into the cell. A FRET signal is generated when donor- and acceptor-labeled tRNAs come in close contact in active ribosomes. The intensity of the FRET signal correlates with the number of ribosomes containing the tRNA FRET pair, providing a cell-based assay for monitoring protein synthesis. PSM can monitor overall protein synthesis, using bulk tRNA, or the synthesis of a specific protein, using one or more specifically selected pairs of tRNA enriched in the protein's sequence; for Collagen I specific isoacceptors of tRNA Proline and Glycine were used.

Results: Using PSM, RNAi and known COL1 inhibitors, we have shown that a specific isoacceptor pair of tRNAgly and tRNApro generates a FRET signal when COL1 is translated in activated human lung fibroblasts. We have developed the assay into a high throughput format, and screened a diverse library of 85K. Subsequently, the specificity of the hits was shown to collagen 1 translation modulation and not as global translation inhibitors. Hits inhibited COL1 accumulation in a dose dpendent and potent manner (EC50<3 uM) and are currently being optimized for activity in a panel of fibrosis relevant assays (lung, liver, Scleroderma) towards the development of a novel treatment for fibrotic diseases.

Conclusions: The novel PSM platform, using collagen I Proline- and Glycine-tRNA pair, revels new set of compounds that selectively inhibits collagen I translation in activated flung fibroblasts. New candidates for antifibrotic drugs could be available after optimizing the leading hit.

Disclosure of Interest: I. Alroy: Employee: Anima Biotech, Ltd., W. Mansour: Employee: Anima Biotech, Ltd., D. Sheppard: Employee: Anima Biotech, Ltd., Z. Smilansky: Employee: Anima Biotech, Ltd., I. Shapira Lots: Employee: Anima Biotech, Ltd.

P04-06YI

The relationship of proinflammatory cytokines, adiponectin with insulin resistance and endothelial dysfunction in patients with NAFLD

Kateryna Pivtorak[1], Iryna Fedzhaga[*2]

[1]Clinical pharmacy and clinical pharmacology, National Pirogov Memorial Medical University Vinnytsya, [2]Internal medicine, Vinnitsia Academy of countinuous education, Vinnitsia, Ukraine

Corresponding author's email: Pivtorakv@gmail.com

Introduction: Recent studies have shown that the unbalanced production of adiponectin and TNF-α contributes to the damage of many tissues, including the liver. Adiponectin counteracts the synthesis of TNF-α, which in turn inhibits the activity of adiponectin.

Aims: The aim - establish a relationship between proinflammatory cytokines and adiponectin with insulin resistance and endothelial dysfunction in NAFLD.

Material and Methods: The study involved 176 NAFLD patients with normal, overweight and obese, and 68 patients with normal, overweight and obese without NAFLD. We determined the level of inflammatory mediator TNF-α, markers (C-reactive protein, fibrinogen), endothelin (ET-1), the activity of the Willebrand factor (vWF), the thickness of the intima-media complex, presence atherosclerotic plaque and stenosis of the carotid arteries, insulin resistance index HOMA-IR for all examined patients. Conducted anthropometric survey, measured levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), the degree of liver fibrosis using elastography (FibroScan), ECG and echocardiography. The stratification of cardiovascular risk was carried by traditional SCORE scale version for countries with high risk.

Results: We found a violation of lipid and carbohydrate metabolism, increased proinflammatory cytokines and decreased adiponectin in NAFLD patients. The concentration of pro-inflammatory cytokines such as TNF-α in patients with NAFLD was 3-7 times higher than the similar parameters of patients with a similar degree of obesity but without evidence NAFLD. Our study showed that the levels of adiponectin were significantly higher in women than men. Levels of adiponectin in serum were related with index HOMA-IR, levels of ET-1, TNF- *α* and GGT, while the level of TNF- *α* was related with, level of ET-1, insulin resistance, level of adiponectin and liver steatosis. The inverse correlation between the levels of adiponectin and liver steatosis degree, TNF - *α* levels and index HOMA-IR was found. The levels of TNF -*α* had a strong direct correlation with steatosis, levels of ET-1 and index HOMA- IR.

Conclusions: Cytokine imbalance and reducing of adiponectin levels contributes to insulin resistance, development of endothelial dysfunction and accumulation of fat in the liver.

Disclosure of Interest: None Declared

P04-08YI

Are clinical profiles of NAFLD in non-obese patients similar to obese patients?

Johnathan H. M. Lum*[1], Jason P. E. Chang[1], Wei Qiang Leow[2], Wei Keat Wan[2], Rafay Azhar[2], Tony K. H. Lim[2], Wan Cheng Chow[1], George B. B. Goh[1]

[1]Gastroenterology & Hepatology, [2]Pathology, Singapore General Hospital, Singapore, Singapore

Corresponding author's email: johnathan.lum@mohh.com.sg

Introduction: Non-alcoholic fatty liver disease (NAFLD) is among the most common chronic liver disease worldwide, and is frequently associated with metabolic syndrome risk factors. However, NAFLD has also been reported in non-obese patients, of which clinical characteristics of biopsy proven cohorts remains to be well elucidated.

Aims: This study aims to analyse patient characteristics of biopsy proven NAFLD in an Asian cohort and explored differences stratified by body mass index (BMI).

Material and Methods: 223 adults with biopsy-proven NAFLD were identified via the institution histology database and enrolled. Clinical information, laboratory variables and histologic data of the whole cohort were characterised. In addition, patients with and without obesity (BMI cut-off 25) were compared.

Results: The cohort consisted of 53.4% male gender with a mean age of 49.9±12.4 years and a mean BMI of 30.7±7.97. Patients with diabetes mellitus, hypertension and dyslipidemia comprised of 48.4%, 60.5% and 65.5% of the cohort respectively.
Histologically, mean NAS was 3.84±1.58 with 61.9% of the subjects having non-alcoholic steatohepatitis (NASH) and 28.7% of them having advanced fibrosis. .
Relative to the non obese subjects, the obese subjects had a lower mean age (48.9 versus 53.2 years, p=0.034) and higher incidence of diabetes (52.9% versus 32.7%, p=0.012). Nevertheless, diabetes control in both groups remained comparable, with similar HbA1c levels (7.72±1.61 versus 6.91±0.89, p=0.08). 42.9% of patients use more than 1 type of diabetes medications and the mean duration of diabetes is 683.5days. Interestingly, prevalence of dyslipidemia (66.1% versus 63.3%, p=0.71), hypertension (64.2% versus 49.0%, p =0.055) and waist circumference (114±16 versus 100±12, p=0.39) were comparable between the two groups. Similarly, the distribution of NASH (61.8% versus 63.3%, p=0.86) and advanced fibrosis (29.2% versus 26.7%, p=0.73) were also not significantly different.

Conclusions: Non-obese patients are equally at risk of developing NASH and advanced fibrosis especially in the context of associated metabolic syndrome risk factors. Factors other than the metabolic components such as genetics and environmental factors may play an important role in fibrogenesis and the pathogenesis of NASH, particularly in the non-obese NAFLD patient. Further in-depth characterisation of the non-obese NAFLD patient is indicated.

Disclosure of Interest: None Declared

P04-09

Circulating long non-coding RNA TCONS_00016452 is inversely correlated with liver steatosis in patients with non-alcoholic fatty liver disease

Jung Gil Park[*1], Soo Young Park[2], Keun Hur[3], Heon Ju Lee[1], Won Young Tak[2], Young Oh Kweon[2], Se Young Jang[2], Yu Rim Lee[2], Gyeonghwa Kim[3], Jihyun Lee[3], Eunhye Lee[3], Hye Won Lee[4]

[1]Internal medicine, Yeungnam University Hospital, [2]Internal medicine, [3]Biochemistry and Cell Biology, Kyungpook National University, [4]Pathology, Keimyung University, Daegu, Korea, South

Corresponding author's email: gsnrs@naver.com

Introduction: Hepatic steatosis is an initial process in patients with non-alcoholic steatohepatitis (NASH), which is progressed to liver fibrosis. Long non-coding RNA (lncRNA) TCONS_00016452 is batch genome conversion of lncRNA *LeXis*, which is related with cholesterol metabolism and hepatic steatosis in mouse.

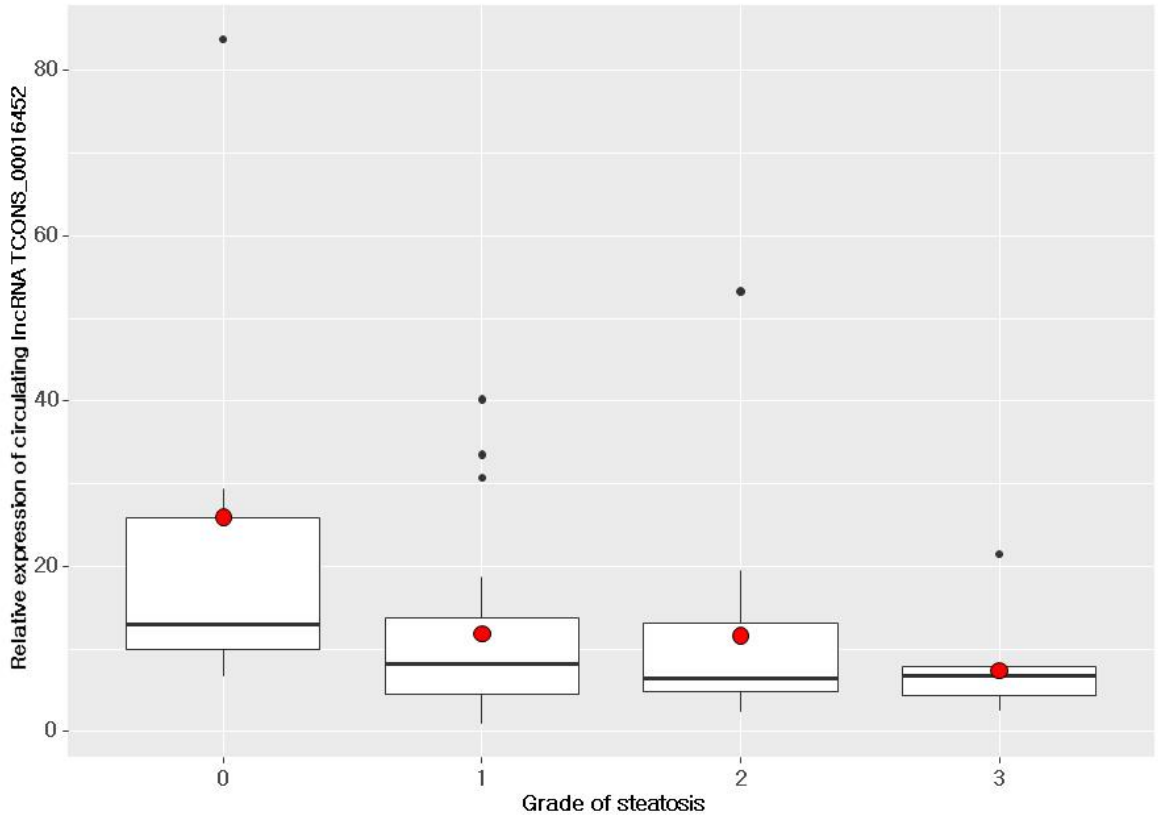
Aims: To evaluate the circulating lncRNA TCONS_00016452 in patients with NAFLD

Material and Methods: We analyzed a total of fifty patients who underwent percutaneous liver biopsy to confirm NASH, which is reviewed by one pathologist. Expression of circulating lncRNA TCONS_00016452 in plasma was compared using quantitative real-time polymerase chain reaction according to pathologic results.

Results: Expression of circulating lncRNA TCONS_00016452 was significantly decreased in patients with severe steatosis compared to patients with minimal to moderate steatosis (13.9±16.0 vs 7.4±5.3, p=0.039. As the grade of steatosis was advanced, circulating lncRNA TCONS_00016452 was decreased (r-value=-0.29, p=0.004). However, circulating lncRNA TCONS_00016452 was decreased in patients with significant fibrosis compare to without significant fibrosis (8.3±5.1 vs 17.2±19.6, p=0.040).

Conclusions: Circulating lncRNA TCONS_00016452 is inversely correlated with grade of liver steatosis in patients with non-alcoholic fatty liver disease.

Figure:



Disclosure of Interest: None Declared

P04-10

Screening of NAFLD using transient elastometry with CAP® and analysis of the component composition of the body to assess the risk of developing liver steatosis

Igor Bakulin[*] [1], Liya N. Belousova[1], Larisa Evdokimova[1], Dmitrii Evdokimov[1], Margarita Serkova[1], Sergei Ivanov[2]

[1]NWSMU named after I.I.Mechnikov, [2]SPbSMU named after I.P. Pavlov, Saint Petersburg, Russia

Corresponding author's email: liya73@mail.ru

Aims: The search for accessible, non-invasive and effective methods of screening for non-alcoholic fatty liver disease (NAFLD), allowing to detect NAFLD at early, potentially reversible stages of development is relevant.
The purpose of the work was frequency estimation of the prevalence steatosis according to elastometry with controlled attenuation parameter (CAP®) among young people and associated body composition.

Material and Methods: 59 volunteers (at the age of 19-28 years (the median age of 20.5) have participated in research. There were 22 (37.3%) men and 33 (62.7%) women among them without verified liver diseases. The survey was conducted in order to exclude or detect risk factors. Determining the presence and degree of steatosis and the stage of liver fibrosis was performed with the FibroScan 502 Touch with CAP® in dB/m was used for the severity of steatosis. The final figures of elasticity of the liver were estimated in kPa (METAVIR). There was the bioelectrical impedance analysis of body (BIA), evaluated: body mass index (BMI), body fat.

Results: The signs of violations of the structure of the liver were diagnosed in 15 people out of 59 (25.4%). The signs of steatosis were founded in 12 (20.3%) students (CAP®>215 dB/m), of liver fibrosis - 7 (11.9%) people (E> 5,8 kPa), combination of liver fibrosis and steatosis was diagnosed in 4 (6.8%). After analyzing data of BIA it was revealed that body weight above normal in 23 (40,3%), wherein fat body composition above normal values in 19 (33,4%). Results of binary regression analysis showed that the chance of development of hepatic steatosis in case of excess adipose tissue increase 28 times (p=0,045), influence of BMI, gender, age was statistically insignificant.

Conclusions: Transient elastography (TE) with controlled attenuation parameter (CAP®) is a fast, reliable, repeatable non-invasive method for the assessment of NAFLD. The development of hepatic steatosis among practically healthy young persons validly associated with the increase the amount of adipose tissue in the body. Confirmed the importance of evaluation of body composition and lack of information of using only BMI when evaluating the chances of development of NAFLD.

Disclosure of Interest: None Declared

P05-01

PNPLA3 but not TM6SF2 polymorphisms were associated with liver fibrosis among Brazilian NAFLD patients

Daniel Ferraz de Campos Mazo[1, 2], Fernanda de Mello Malta[1], Jose Tadeu Stefano[1], Ana Paula Moreira Salles[1], Michele Soares Gomes-Gouvea[1], Jazon Romilson de Souza Almeida[2], Joao Renato Rebello Pinho[1], Flair Jose Carrilho[1], Claudia P. Oliveira[*1]

[1]gastroenterology - Lim 07, University Of Sao Paulo School Of Medicine, Sao Paulo, [2]Department of Gastroenterology,, University of Campinas, Campinas, Brazil., Campinas, Brazil

Corresponding author's email: claudia.oliveira220@fm.usp.br

Introduction: In recent years, genome-wide association studies have shown that two single-nucleotide polymorphisms (SNPs) PNPLA3 and TM6SF2 were associated with steatosis and progression of NAFLD in different populations.

Aims: We aimed to evaluate PNPLA3 and TM6SF2 SNPs in healthy Brazilian subjects in comparison to NAFLD patients, and evaluate its associations with hepatic steatosis and severity of liver disease.

Material and Methods: We performed a multicenter cross-sectional study in healthy controls and biopsy-proven NAFLD patients followed at the Outpatient Liver Unit of the Department of Gastroenterology of the University of Sao Paulo and at the Division of Gastroenterology of the University of Campinas, Brazil. We evaluated PNPLA3 (rs738409 c.444 C>G) and TM6SF2 (rs58542926 c.449C>T) SNPs frequency and possible associations with age, body mass index, laboratory tests and liver histology (inflammation, ballooning, steatosis and fibrosis) among nonalcoholic steatohepatitis (NASH) patients. P< 0.05 was considered significant.

Results: 134 healthy controls and 248 NAFLD patients were included (34 with steatosis and 214 with NASH). In control group, the frequencies of PNPLA3 CC and CG+GG were 49.25% and 50.74%, respectively, and in NAFLD patients were 31.05% and 68.88%, p= 0.0044. For TM6SF2, the frequencies of CC and CT+TT in control group were 93.28% and 6.72%, respectively, and among NAFLD patients were 89.47% and 10.53%, p= 0.0821. After adjusting for age and gender on logistic regression analyses, PNPLA 3 GG subjects had and increased risk of 3.29x of having NAFLD when compared to CC subjects (p=0.0132, CI 1.641-8.224). These SNPs were unable to predict NASH among NAFLD patients (p=0.175). Specifically in NASH patients, PNPLA3 GG compared to CC was associated with higher AST levels [38.4±25.3U/L *vs* 36.7±40.1IU/L, p=0.0395)] and the presence of significant liver fibrosis (≥F2 Metavir fibrosis score, p=0.0272). No differences were observed in the supra-cited variables among NASH patients regarding TM6SF SNPs.

Conclusions: We demonstrated for the first time that PNPLA3 C/G+GG increase the risk of NAFLD among Brazilian subjects. Moreover, PNPLA3 allele G is associated with liver enzyme elevation and fibrosis in Brazilian biopsy-proven NAFLD patients, as observed in other populations. Although TM6SF2 SNPs were not associated with liver disease severity, further studies are necessary to evaluate the role of the combination of both SNPs in Brazilian NAFLD patients.

Disclosure of Interest: None Declared

P05-02

SNP-630, a novel inhibitor of fatty acid omega-oxidation, reverses non-alcoholic steatohepatitis-derived liver injury and fibrosis in a high-fat-diet-fed murine model

Hsin-Tien Ho[*1], Kai-Min Chu[1], Cheng-Huei Hsiong[1], Yung-En Wu[1], Chen-Che Su[1], Jia-Yu Hao[1], Oliver Yoa-Pu Hu[2,3]

[1]Sinew Pharma Inc, [2]Taipei Medical University, [3]National Defense Medical Center, Taipei, Taiwan

Corresponding author's email: hth@sinewpharma.com

Introduction: Non-alcoholic steatohepatitis (NASH) is considered an important factor that leads to liver damages such as severe liver fibrosis, cirrhosis, and even liver cancer. However, no approved drug provides specific therapeutic effects on NASH. Activation of fatty acid omega-oxidation-related enzymes such as cytochrome P450 may be attributed to the pathological mechanism of NASH.
Aims: The aim of the study was to determine if administration of a novel synthetic omega-oxidation-related enzyme inhibitor, SNP-630, would ameliorate liver injury and fibrosis in a murine model of NASH.

Material and Methods: SNP-630, a new synthetic molecule designed to inhibit fatty acid omega-oxidation-related enzymes, was chosen to test the anti-NASH effect in a standard animal model. Male C57BL/6 mice were fed regular chow (RD) or high-fat diet (HFD) for 20 weeks, following which, they were co-administered with vehicle or SNP-630 by oral gavage once daily for another 10 weeks. Serum biochemistry, hepatic lipids, and organ histopathology were evaluated. Omega-oxidation-related enzyme inhibitory assays were assessed in a separate study. Furthermore, we conducted a clinical trial for two active metabolites of SNP-630 to evaluate their tolerability, safety, and pharmacokinetics in health volunteers.

Results: The mice that were fed HFD demonstrated an 18.2-fold increase in serum alanine aminotransferase (ALT) levels and a 20.1-fold increase in hepatic triglyceride content, respectively. These increases were substantially attenuated in the mice treated with SNP-630 (HFD-S) in a dose-dependent manner. Likewise, liver steatosis and inflammation were reduced in the mice treated with HFD-S as compared with those treated with HFD, on the basis of the non-alcoholic fatty liver disease activity score histological scores. The mice treated with SNP-630 also showed a marked reduction in liver fibrosis as determined using Masson's trichrome stain. In a human study, no drug-related adverse events caused by the two active metabolites of SNP-630 were reported, and the preliminary safety data of its active metabolites were guaranteed.

Conclusions: In conclusion, the data presented in this report demonstrate that hepatic steatosis, NASH injury, and fibrosis could be suppressed by inhibiting the activation of omega-oxidation-related enzymes in a murine model of NASH. To the best of our knowledge, SNP-630 is the most potent candidate for further demonstration of its efficacy and safety in the following clinical trials.

Figure:

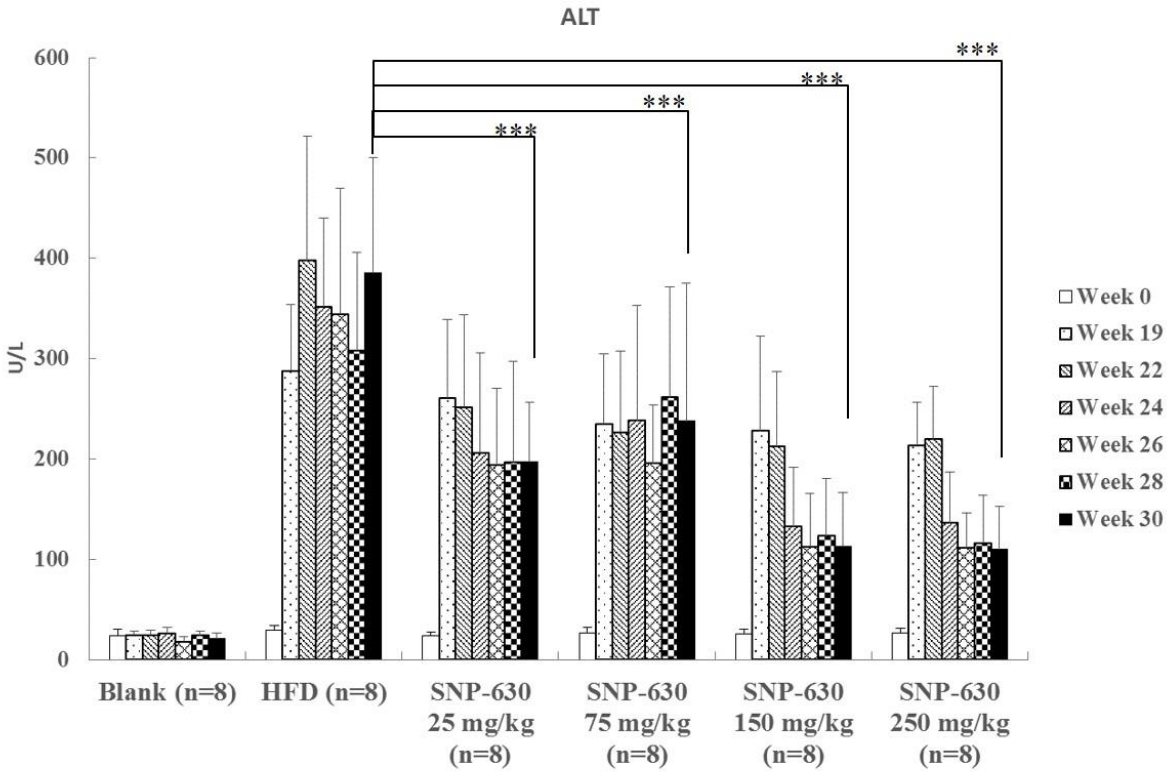


Fig. 1 SNP-630 could reduce liver dysfunction in a murine model of NASH.
Mice fed either a normal chow diet (blank group) or a HFD (60% of calorie from fat; HFD and SNP-630 groups) for 30 weeks. Oral SNP-630 were co-administered with HFD from week 21 to 30 for SNP-630 groups. Statistical differences are presented as ANOVA and LSD. ***$p<0.005$, compared to the HFD group.

Disclosure of Interest: H.-T. Ho: Sponsored lectures (National or International): Sinew Pharma Inc, Stockholder: Sinew Pharma Inc, Employee: Sinew Pharma Inc, K.-M. Chu: Sponsored lectures (National or International): Sinew Pharma Inc, Stockholder: Sinew Pharma Inc, Employee: Sinew Pharma Inc, C.-H. Hsiong: Sponsored lectures (National or International): Sinew Pharma Inc, Stockholder: Sinew Pharma Inc, Employee: Sinew Pharma Inc, Y.-E. Wu: Sponsored lectures (National or International): Sinew Pharma Inc, Employee: Sinew Pharma Inc, C.-C. Su: Sponsored lectures (National or International): Sinew Pharma Inc, Employee: Sinew Pharma Inc, J.-Y. Hao: Sponsored lectures (National or International): Sinew Pharma Inc, Employee: Sinew Pharma Inc, O. Y.-P. Hu: Consultant: Sinew Pharma Inc

P05-03YI

Angiopoietin-2 as therapeutic target for pathological angiogenesis and inflammation in the pathogenesis of non-alcoholic steatohepatitis

Sander Lefere[1], Sarah Raevens[1], Sara Neyt[2], Christian Vanhove[3], Christophe Casteleyn[4], Xavier Verhelst[1], Hans Van Vlierberghe[1], Anja Geerts[1], Lindsey Devisscher[*1]

[1]Department of Gastroenterology and Hepatology, Hepatology Research Unit, Ugent Belgium, [2]Molecubes NV, [3]Institute Biomedical Technology, Medical Imaging and Signal Processing, [4]Department of Morphology, Faculty of Veterinary Medicine, Ugent Belgium, Ghent, Belgium

Corresponding author's email: Lindsey.devisscher@ugent.be

Introduction: Non-alcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease and covers a spectrum from simple steatosis to non-alcoholic steatohepatitis (NASH), the most severe form of NAFLD. To date, only life style management is effective and no pharmacological treatment options are available.
Angiogenesis and inflammation play an important role in the progression of NAFLD to NASH and angiopoietin-2 (Ang-2) mediates angiogenesis and inflammation.

Aims: Our aim was to investigate the role of Ang-2 and its potential as therapeutic target in NASH by using human samples, a mouse model and *in vitro* assays.

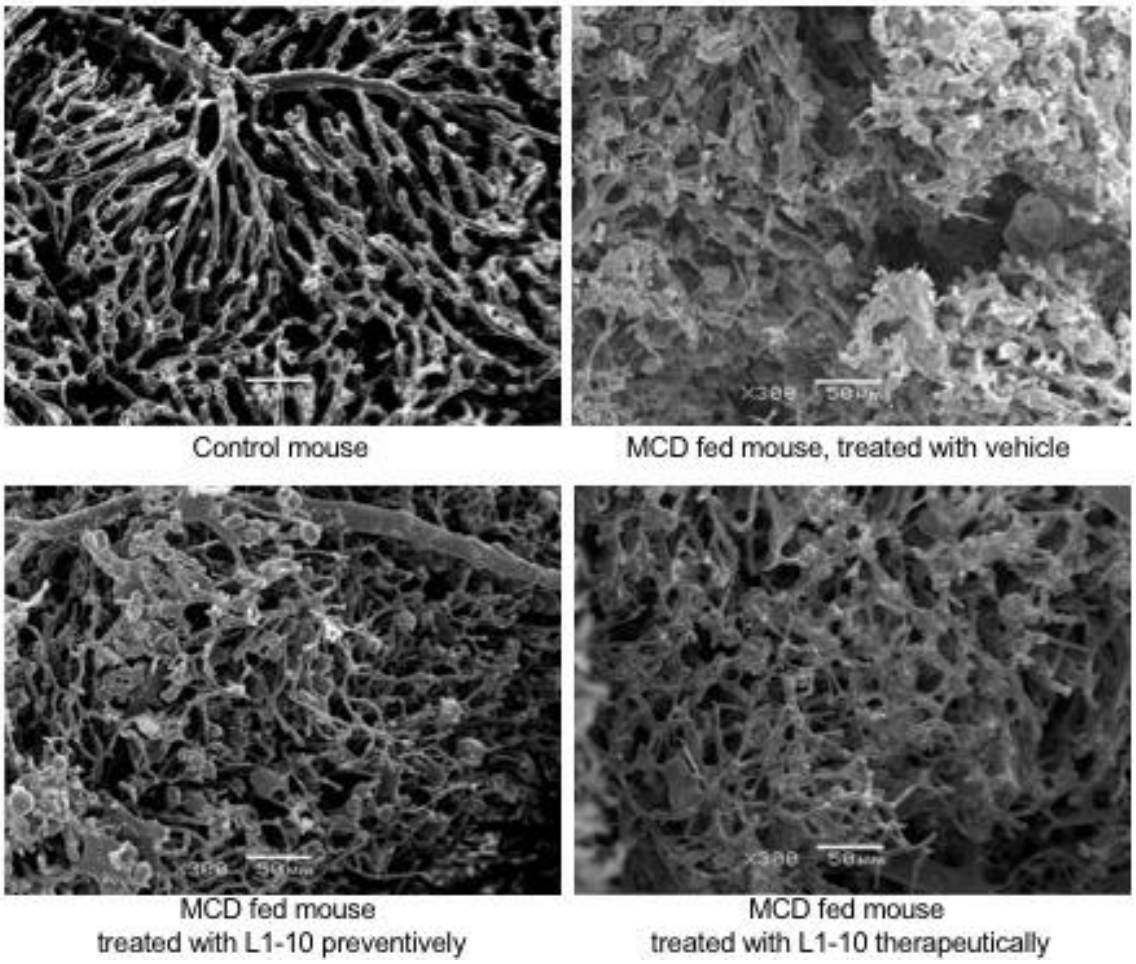
Material and Methods: Serum Ang-2 levels were determined in obese patients and in methionine choline deficient (MCD) diet fed mice. The Ang-2 inhibitor L1-10 (Amgen) was tested *in vivo* in the MCD diet model and *in vitro* on bone-marrow derived macrophages and endothelial MS1 cells. NAFLD activity score was analysed on histology. The hepatic vascular bed was visualized using corrosion casts and contrast-enhanced microCT (X-CUBE, MOLECUBES). FACS-isolated liver endothelial cells and monocytes were analyses by qRT-PCR. *In vitro* cytokine secretion and phenotypes were analysed by luminex and ELISA respectively.

Results: Serum Ang-2 levels were increased in patients with NASH compared to lean controls, obese patients without NAFLD and patients with simple steatosis (all $p<0.01$) and correlated with the severity of steatosis ($p<0.05$)**,** inflammation and ballooning (both $p<0.001$) but not fibrosis. In line, serum and liver Ang-2 levels were increased in MCD fed mice ($p<0.001$). MCD fed mice treated with L1-10 showed less inflammatory foci and ballooning hepatocytes compared to untreated MCD fed mice ($p<0.05$). Electron microscopy of vascular corrosion casts of MCD fed mice showed an irregular vessel pattern which was less pronounced in L1-10 treated mice and microCT confirmed reduced vascular bed expansion in L1-10 treated mice. Liver endothelial cells and monocytes from L1-10 treated MCD fed mice expressed lower levels of dysfunction and inflammatory markers compared to cells from control treated mice. LPS stimulated bone-marrow derived macrophages and MS1 cells secreted pro-inflammatory cytokines and chemokines and showed increased expression of endothelial dysfunction markers which was significantly less pronounced in cells treated with LPS + L1-10.

Conclusions: Our findings provide evidence for Ang-2 inhibition as therapeutic strategy to target pathological angiogenesis and inflammation in NASH.

Figure:

Scanning electron microscopy of liver vascular corrosion casts of control and MCD fed mice, treated with the ang-2 inhibitor L1-10 or vehicle.



Disclosure of Interest: None Declared

P05-04

Advanced non-alcoholic fatty liver disease (NAFLD) modeling using human liver 3D extracellular matrix scaffolds

Lisa Longato[*1,2], Giuseppe Mazza[1,2], Walid Al Akkad[1,2], Arul Madhavan[2], Andrea Telese[2], Luca Frenguelli[2], Andrew R. Hall[2], Krista Rombouts[1,2], Massimo Pinzani[1,2]

[1]Engitix Limited, [2]Institute for Liver and Digestive Health, University College London, London, United Kingdom

Corresponding author's email: lisa.longato@engitix.com

Introduction: Due to its increasing global prevalence and the significant morbidity associated with it**,** NAFLD is a condition that represents a public health concern worldwide, for which no approved pharmacological therapies exist. The development of novel platforms for disease modeling and drug testing could be an important addition to existing animal and *in vitro* 2D models to help developing new drugs for NAFLD.

Aims: This study was aimed at developing a novel 3D culture system to model NAFLD in vitro, utilizing human acellular biological liver scaffold.

Material and Methods: Liver tissue cubes were obtained from donor liver tissue unsuitable for transplantation, sectioned into 125 mm^3 cubes and decellularised to obtain intact hepatic extracellular matrix 3D scaffolds, using a proprietary method recently developed. Cell removal and integrity of the matrix were confirmed by DNA quantification and immunohistochemistry. Steatotic 3D cultures were obtained by repopulation of scaffolds with HepG2 human hepatoma cells for 7 days, then treatment for additional 7 days with a non-cytotoxic dose of palmitic (PA) or oleic acid (OA). The resulting 3D cultures were analyzed for morphological changes, extent of steatosis via Oil Red O staining, and gene expression analysis for markers of lipid metabolism. 3D cultures were compared to 2D cultures similarly treated.

Results: Prolonged culture of HepG2 cells in low-serum medium was well tolerated, and exposure to a 100 µM FA dose was non cytotoxic and effective at inducing steatosis in both 2D and 3D cultures. OA treated 3D cultures were characterized by a more robust and macrovesicular steatosis, compared to PA. Importantly, treatment with either PA or OA in 3D cultures induced changes in several biochemical pathways of lipid metabolism, including lipid droplets markers (PLIN2), fatty acids synthesis (SCD-1), and catabolism (CPT1α). Notably, baseline gene expression was largely different between 2D and 3D cultures.

Conclusions: This pilot study demonstrates the feasibility of the development of a 3D NAFLD model using human biological scaffold. These findings confirm the importance of the substrate in which the cells are grown in modulating cell behaviour. The potential advantages of such novel 3D model include the use of human cells/scaffolds, extended duration of cultures and treatments, higher biological relevance of cells grown in 3D, and the potential for ease of incorporation of multiple cell types to generate co-cultures.

Disclosure of Interest: None Declared

P05-05

Suppression of migration and proliferation of hepatocellular carcinoma cells by Poncirus Fructus via Inhibition of PIN1

Lokendra Chand*[1], Suvesh Munakarmi[1], Yeon J. Jeong[2]

[1]Biomedical Research Institute, Chonbuk National University, [2]Department of Surgery, Chonbuk National University Hospital, Jeonju, Korea, South

Corresponding author's email: chandlb76@jbnu.ac.kr

Introduction: Hepatocellular Carcinoma (HCC) is one of the most common carcinoma and leading cause of cancer related death world-wide. Many epidemiological studies have demonstrated that patients with HBV infection have significantly higher rate of hepatocarcinogenicity. HCC is emerging challenge which is arising increasingly in precirrhotic stages. There are number of drugs that are already in developmental pipeline, none of them are as yet, approved by regulatory agencies. Poncirus Fructus (PF) is a phytochemical found in dry, immature fruits of *Poncirus trifoliata*, traditionally used for the treatment of gastrointestinal disorders and blood circulation related disease in East Asia, can be the potential candidate for the treatment of HCC. This study demonstrated that the expression of PIN1 is inhibited by PF. PIN1 is parvulin family member of PPIase enzyme plays the important role in cell signaling by binding with the specific motifs comprising p-Ser/The-pro residue via *cis-trans* isomerization and overexpressed in HCC contributing hepatocarcinogenesis.

Aims: The aim of this study is to find out the role of PF in the treatment of HCC with Hep3B cell line.

Material and Methods: Hep3B and AML-12 cells were cultured in DMEM medium with FBS and antibiotics. Cell viability was evaluated by MTT assay. Cell migration was examined by performing wound healing assay. Expression of PIN1, apoptosis, cell cycle and inflammation marker were examined by western blotting analysis.

Results: The cell viability assay shows that the number of viable Hep3B cells was found to be decreased while treated with PF in the dose dependent manner but the AML-12 cells remains unaffected. PF induces the apoptosis, downregulates cell cycle and inflammation markers and downregulates the expression of PIN1 in Hep3B cells but no such expression was examined in AML-12 cells, which implies that the proliferation of Hep3B cells was inhibited and cell death is enhanced by the PF.

Conclusions: In conclusion, PF suppresses the migration and proliferation of HCC cells by the inhibition of PIN1 without affecting the normal cells.

Disclosure of Interest: None Declared

P05-06

Nicotinamide ameliorates hepatic steatosis via sirtuin activation

Makoto Nakamuta*[1], Eiji Mita[2], Shinichi Kiso[3], Kenji Ohe[4], Munechika Enjoji[4]

[1]Gastroenterology, National Hospital Organization Kyushu Medical Center, Fukuoka, [2]Gastroenterology and Hepatology, National Hospital Organization Osaka National Hospital, Oska, [3]Community Medicine, Osaka Universit, Graduated School of Medicine, Osaka, [4]Clinical Pharmacology, Fuculty of Pharmaceutical Science, Fukuoka University, Fukuoka, Japan

Corresponding author's email: nakamuta@kyumed.jp

Introduction: Sirtuins (Sirts), the so-called NAD^+-dependent deacetylases, are involved in protection from hepatic steatosis as well as longevity. Nicotinamide (NAM) is a metabolite from NAD catalyzed by Sirts, and is reutilized for NAD synthesis through nicotinamide mononucleotide (NMN) produced by nicotinamide phosphoribosyltransferase (Nampt) (Fig 1). NAM is also converted to N^1 methylnicotinamide (MNAM) by nicotinamide N-methyltransferase (Nnmt). MNAM was reported to improve hepatic steatosis through Sirt1 protein stabilization (Nat Med. 2015;21:887). On the other hand, the knockdown of Nnmt was reported to protect the liver from hepatic steatosis (Nature. 2014;508:258).

Aims: We were motivated to investigate this yet to be decifered issue of whether NMA has a protective effect on hepatic steatosis.

Material and Methods: C57BL/6J mice (n=20) were divided into four groups and fed with normal diet (ND), ND+NAM (NAM mixed with ND to 0.1% wt/wt), high fat diet (HFD), or HFD+NAM for 8 weeks. The contents of NAM and MNAM in the liver and serum were measured by LC/MS. The expression of NAD metabolism-related genes was evaluated by real-time RT-PCR.

Results: NAM prevented HFD-induced weight gain, and there was no significant difference in body weight among ND, ND+NAM, and HFD+NAM. NAM also histologically ameliorated hepatic steatosis caused by HFD. NAM dramatically increased the serum NAM concentration both in ND+NAM and HFD+NAM, but did not increase the hepatic NAM contents (Fig 2A). Interestingly, in MNAM, the hepatic and serum MNAM levels were increased by NAM to ND, whereas there was no difference in the levels between HFD and HFD+NAM (Fig 2B). Furthermore, the gene expression of Nnmt was significantly decreased in ND+NAM, HFD, HFD+NAM, and that of Nampt was increased only in HFD+NAM, when both compared to ND. These results indicated that, in ND+NAM, the supplemented NAM mainly converted into MNAM; in contrast, in HFD+NAM, NAM converted into NMN rather than MNAM to supply NAD. Indeed, NAM enhanced the expression of Sirt 3, 4, and 5 known to be involved in lipid metabolism such as beta oxidation in the mitochondria. In HFD+NAD, NAM enhanced the expression of beta oxidation-related genes; oppositely, it suppressed that of fatty acid synthesis-related genes.

Conclusions: NAM ameliorated hepatic steatosis through the activation of Sirts, leading to enhanced fatty acid oxidation and suppressed fatty acid oxidation. Our results suggest that NAM might have a novel therapeutic application for NAFLD/NASH.

Figure:

Figure 1

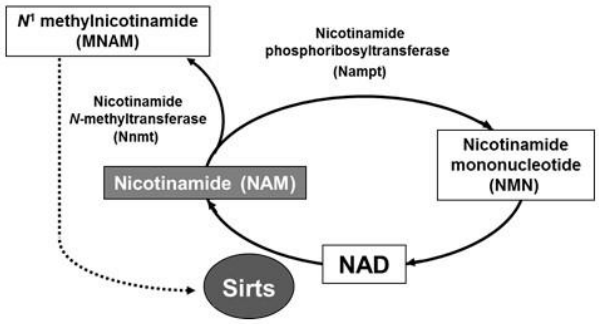
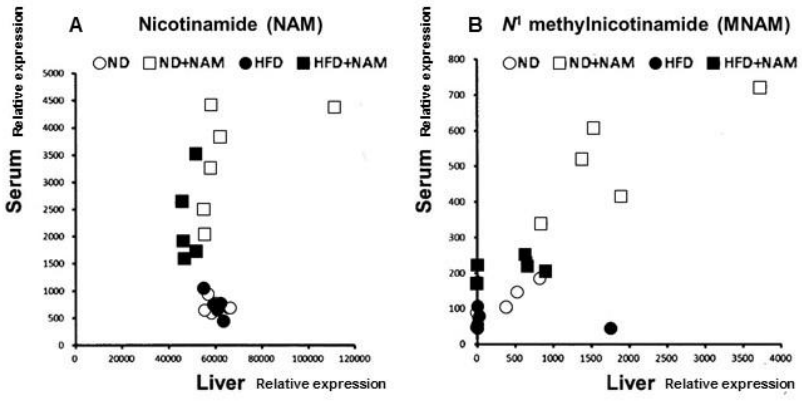


Figure 2



Disclosure of Interest: None Declared

P05-07YI

Bisphenol A as a trigger of inflammation in non-alcoholic fatty liver disease: in vitro and in vivo study

Marcello Dallio[*] [1], Giuseppe G. Caprio[1], Mario Masarone[2], Sonia Errico[3], Carla Nicolucci[3], Marcello Persico[2], Nadia Diano[3], Carmelina Loguercio[1], Alessandro Federico[1]

[1]Department of Clinical and Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, [2]Department of Internal Medicine and Hepatology, University of Salerno, Baronissi, [3]Department of Experimental Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy

Corresponding author's email: marcello.dallio@gmail.com

Introduction: Bisphenol A (BPA) is an endocrine disrupting chemical found in food packages or insecticide residues on vegetable crops, associated with type 2 diabetes mellitus (T2DM), cardiovascular disease and liver enzyme abnormalities.

Aims: We evaluated BPA plasma and urine levels in non-alcoholic fatty liver disease (NAFLD) patients compared to healthy subjects. Furthermore, we evaluated, in human HepG2 cells, the effects of exposure to different concentrations of BPA on both oxidative stress induction and cell proliferation.

Material and Methods: We enrolled sixty patients with histological diagnosis of NAFLD with or without T2DM and sixty healthy subjects. In vitro, the proliferation of BPA-exposed HepG2 cells at two different concentrations (0.025 and 0.05µM) was evaluated, both at high (H-HepG2) and at low (L-HepG2) glucose concentrations for 48h. Lipoperoxidation was assessed by thiobarbituric acid reactive substances (TBARS) assay.

Results: BPA levels were significantly higher in 60 NAFLD subjects, both in urine and in plasma ($p<0.0001$) if compared to controls and, in this group, it appeared to be higher in 30 non-alcoholic steatohepatitis patients compared to 30 simple steatosis ones ($p<0.05$), independently from the presence of T2DM.
After a BPA-free diet for one month, NAFLD patients showed a significant reduction of BPA circulating levels ($p<0.05$), without a significant reduction of urine levels.
H-HepG2 cells treated with BPA (0.05 µM) increased proliferation compared to controls at 48h ($p<0.0001$). BPA increased TBARS levels at 48h versus controls.

Conclusions: Our study reveals a possible role of BPA as environmental factor involved in the promotion of NAFLD, particularly in T2DM patients.

Disclosure of Interest: None Declared

P05-08

Lipidomic profiling and liver tissue autofluorescence of fatty livers correlate with the organ susceptibility to cold storage preservation

Andrea Ferrigno[1], Clarissa Berardo[1], Laura G Di Pasqua[1], Veronica Siciliano[1], Vittoria Rizzo[2], Barbara Mannucci[3], Plinio Richelmi[1], Giuseppina Palladini[1], Anna Cleta Croce[4], Mariapia Vairetti[*1]

[1]Dept Internal Medicine and Therapeutics, [2]Dept Molecular Medicine, Fondazione IRCCS Policlinico S.Matteo, [3]Centro Grandi Strumenti, University of Pavia, [4]Institute of Molecular Genetics, Italian National Research Council (CNR), Pavia, Italy

Corresponding author's email: mariapia.vairetti@unipv.it

Introduction: Although fatty livers are more prone to primary dysfunction and nonfunction after transplantation, they represent a possibility to reduce the discarded organs. Animal models of hepatic steatosis are essential to understand the mechanisms underlying preservation injury of graft under hypothermic conditions. Liver tissue autofluorescence (AF) is an innovative resource to monitor in situ fluorescing fatty acids.

Aims: In this study, we investigated how the lipidomic profile correlates with the liver susceptibility to cold storage preservation using two rat models of NAFLD: the methionine and choline deficient (MCD) diet and the Obese Zucker fa/fa rats.

Material and Methods: NAFLD was induced in male Wistar rats by 2-week MCD diet; 12-week male old obese (fa/fa) Zucker rats were also used. Isolated livers were preserved in UW solution at 4°C for 6 hours (Cold Storage, CS) followed by reperfusion (2 hours). Serum and biliary enzyme release, bile production, portal pressure, tissue glutathione (GSH) and lipid peroxides were evaluated. Before preservation, liver fatty acid (FA) profiling was performed by mass spectrometry (MS) and parallel estimation of fluorescing fatty acids (FFA) by fitting analysis of AF spectra recorded under 366 nm excitation.

Results: According to the FA analysis by MS, in Zucker and MCD rats the total saturated/polyunsaturated fatty acid (PUFA) ratio was 1.5 and 0.71, respectively. In MCD rats showed a three-time decrease in saturated stearic acid, an eight-time increase in polyunsatured linoleic acid and a three-time decrease in polyunsatured arachidonic acid. FFA data by AF fully reflected the changes observed by means of MS in linoleic and arachidonic acid levels. After 6 hours CS and 2 hours reperfusion, a three-time increase in AST, two-time increase in biliary g-GT, lower bile production and a marked rise of portal pressure were found in Zucker obese rats versus MCD group. No difference in tissue GSH levels and increase in lipid peroxides were found in the MCD group versus Zucker obese rats.

Conclusions: Our results suggest that cellular injury is strongly associated with the liver composition in FAs, which represent an effective prognostic target; fatty livers from Zucker obese donors characterized by higher levels of saturated FA, are more prone to CS injury as compared with MCD livers with high PUFA content. Supported by MS analysis, the ability of AF to promptly reveal the liver lipidomic profiling can be proposed for the prescreening donor livers.

Disclosure of Interest: None Declared

P05-09

Development of in vitro 3D NAFL/NASH models with primary human hepatocytes, hepatic stellate cells and Kupffer cells

Marion Duriez[*1], Anne-Céline Le Fèvre[1], Fanny Volat[1], Xavier Vigé[1], Jean-Claude Guillemot[1], Aimo Kannt[2], Cécile Orsini[1], Vincent Mikol[1] and on behalf of the PreciNASH Consortium

[1]Translational Sciences, Sanofi R&D, Chilly-Mazarin, France, [2]R&D Diabetes, Sanofi, Frankfurt, Germany

Corresponding author's email: marion.duriez@sanofi.com

Introduction: Non-alcoholic fatty liver disease (NAFL) includes a broad spectrum of pathologies from simple steatosis to more severe non-alcoholic steatohepatitis (NASH) and cirrhosis. Mechanisms involved in NAFL/NASH disease progression are still misunderstood. Beside hepatocytes, both Hepatic Stellate cells (HSC) and Kupffer cells (KC) play an important role in the pathogenesis-related fibrosis and inflammation.

Aims: The objective of this work is to design *in vitro* 3D human liver models to investigate mechanisms involved in NAFL/NASH disease progression.

Material and Methods: *In vitro* 3D liver models were setup using collagen-based hydrogel and primary human hepatocytes (PHH), HSC and KC. Cell viability, steatosis, fibrosis and inflammation will be evaluated.

Results: PHH were cultured *in vitro* in 3D up to 21 days and the 3D hydrogel was optimized. PHH viability, apoptosis, polarization and albumin secretion were monitored. Medium mimicking stages of NAFL/NASH disease using different free fatty acid compositions and concentrations were tested. Intracellular lipid droplets were imaged by confocal microscopy and quantified. Triglyceride and cholesterol synthesis by PHH were also assessed.
HSC and KC viabilities and phenotypes in our selected NAFL/NASH culture medium were investigated and *in vitro* 3D PHH/HSC and KC co-cultures are currently evaluated. HSC and KC activation reflecting respectively fibrosis and inflammation will be addressed.

Conclusions: *In vitro 3D* human liver NAFL/NASH models are promising tools to decipher mechanisms involved in NAFL/NASH disease progression and are of high interest for drug screening.

Disclosure of Interest: None Declared

P05-10

Metabolomics – A new approach to the diagnosis of NAFLD

Igor Bakulin*[1], Liya Belousova[1], Madina Abatsieva[2]

[1]North-Western state medical University named after Mechnikov, Saint Petersburg, [2]Северо-западный государственный медицинский университет им. Мечникова, Санкт-Петербург, Russia

Corresponding author's email: igbakulin@yandex.ru

Introduction: Regarding "Omic" technologies, modern achievements to date have been promising. Metabolomics is the youngest science and represents a complete set of low-molecular compounds. Diseases of various organs and systems are accompanied by changes that resulted in the creation in various tissues and biological fluids of certain metabolites that reflect functional changes and serve as biochemical markers. Determination of metabolites allows you to monitor the functional State, track the changes in basic systems, screen pathological changes, as well as identify the effectiveness and efficiency of the therapy.

Aims: to examine data about the potential of "omic" technologies in the diagnosis of NAFLD.

Material and Methods: using databases (Pubmed, Scopus) to analyse the study, which examines the metabolom profile in diseases of the digestive tract.

Results: Currently showing opportunities identification NAFLD potential biomarkers metabolomics, NASH, 2010 study (United States) 437 evaluated various metabolites in serum of patients with NAFLD using ZhH-MS and GC-MS, found that in patients with NASH level of free long-chain fatty acids acids were significantly lower compared to the control group. LoombaR.et al. (2012) explored the importance of polyunsaturated fatty acids as arachidonic, in particular diagnostic biomarkersof non-invasive diagnosis NASH: 11.12-diHETrE, DHK PGD, 20 2-COOH AA were significantly higher in the Group of patients with NASH versus NAFLD. In 2016g. A. Feldman et demonstrated that NAFLD in patients with BMI ≥ 25 and obese patients have a different profile of amino acids and acyl-carnitine. When NAFLD was given to a group with normal BMI, the result were: lower concentrations of sintez-phosphatidylcholine, alanine, tyrosine and valine and higher lysine concentration in comparison to people with obesity.

Conclusions: assessment of fatty acids as new biomarkers NAFLD and NASH can be used as a differential diagnostic marker and predictor of progressive currents NAFLD in general. In particular, using GC-MS in the determination of serum fatty acids reduces analysis time, increases its accuracy and reliability in various stages of NAFLD whilst helping clinicians with diagnosis and evaluation of prediction.

Disclosure of Interest: None Declared

P06-01YI

RIP3-dependent signalling contributes to non-alcoholic fatty liver disease-related carcinogenesis

Marta B. Afonso[*1], Pedro M. Rodrigues[1], André L. Simão[1], Maria M. Gaspar[1], Rui E. Castro[1], Cecília M. P. Rodrigues[1]

[1]Research Institute for Medicnes (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Corresponding author's email: mbafonso@ff.ulisboa.pt

Introduction: Hepatocellular death, inflammation and fibrosis are implicated in the pathogenesis of non-alcoholic steatohepatitis (NASH), including development and progression to hepatocellular carcinoma (HCC). Necroptosis is a highly immunogenic regulated cell death routine that depends on receptor-interacting protein 3 (RIP3) kinase activity.

Aims: We aimed to evaluate the role of necroptosis in the pathogenesis of non-alcoholic fatty liver disease (NAFLD)-driven carcinogenesis.

Material and Methods: C57BL/6 wild-type (WT) or RIP3-deficient ($RIP3^{-/-}$) mice were fed a choline-deficient L-amino acid-defined diet (CDAA; n=14) or a control choline-sufficient L-amino acid-defined (CSAA; n=14) diet for 66 weeks, with subsequent histological and biochemical analysis of hepatic damage and carcinogenesis. Insulin resistance and oxidative stress were also investigated.

Results: CDAA-fed WT mice exhibited all the main histological features of liver injury associated with NASH, namely steatosis, hepatocellular ballooning, immune cell infiltration, and fibrosis. RIP3 deficiency ameliorated CDAA-induced inflammation and fibrosis, and decreased the NAFLD activity score. In agreement, hepatic gene expression of pro-inflammatory mediators was also significantly decreased in CDAA-fed $RIP3^{-/-}$ mice, compared with WT. Intriguingly, $RIP3^{-/-}$ mice displayed increased body weight gain with time, as well as insulin resistance at 66 weeks as assessed by homeostasis model assessment-estimated insulin resistance and decreased insulin receptor substrate phosphorylation, compared with WT mice on CSAA or CDAA diet. $RIP3^{-/-}$ mice tended to show reduced incidence of macroscopic preneoplasic nodules, accompanied by significantly reduced Ki67 positive hepatocytes and increased proapoptotic Bax and cell cycle regulator cyclin-dependent kinase 2-associated protein 1 (CDK2AP1). Absence of RIP3 further hampered signaling pathways controlling tumor microenvironment and protected against oxidative stress and mitochondrial dynamic dysfunction.

Conclusions: Overall, RIP3 ablation halts long-term inflammation, fibrosis, hepatocyte proliferation, and genetic resistance of dysplastic hepatocytes to cell death, oxidative stress and tissue microenvironment changes associated with NASH-driven hepatocarcinogenesis. Targeting RIP3-dependent signalling might be a promising approach to arrest NAFLD progression to HCC, although complementary approaches may be required to control insulin resistance in obese patients. (Funding: PTDC/BIM-MEC/0895/2014; SAICTPAC/0019/2015).

Disclosure of Interest: None Declared

P06-02YI

Liver transient elastography in non-alcoholic fatty liver disease: is there any predictive role in the development of colorectal polyps?

Marta Gravito-Soares[*1], Elisa Gravito-Soares[1], Dário Gomes[1], Adélia Simão[2], Luis Tomé[1] and Faculty of Medicine, Coimbra University and Gastroenterology Department, Centro Hospitalar e Universitário Coimbra, Coimbra, Portugal

[1]Gastroenterology, [2]Internal Medicine, Centro Hospitalar e Universitário Coimbra, Coimbra, Portugal

Corresponding author's email: ms18498@gmail.com

Introduction: Recent studies have demonstrated an association between abnormal glycemia, dyslipidemia and metabolic syndrome, and an increased risk of colorectal polyps. Patients with non-alcoholic fatty liver disease (NAFLD) often have these risk factors. The association between NAFLD and colorectal polyps has been poorly studied.

Aims: To evaluate the prevalence and risk factors of colorectal polyps in patients with NAFLD.

Material and Methods: Retrospective observational cohort study of 237 patients with NAFLD underwent transient elastography by Fibroscan®, between 01/2015-02/2017. Exclusion criteria: age<18years, absence of total colonoscopy with good preparation <3 years, inflammatory bowel disease, hereditary polyposis syndromes and personal/family history of colorectal polyps/neoplasia. Compared patients with colorectal polyps (cases) and without colorectal polyps (controls). Demographic variables, cardiovascular/metabolic risk factors, comorbidities, laboratory parameters and Fibroscan® *scores* of steatosis (CAP>300dB/m) and fibrosis (F4:>10KPa) were evaluated.

Results: Of the 237 NAFLD patients who performed Fibroscan®, 103 underwent total colonoscopy. The prevalence of colorectal polyps was 28.2%(n=29):19.4% hyperplastic, 16.5% adenoma and 4.8% advanced adenoma/adenocarcinoma. The mean age was 58.32±51years (vs57.09±10.53;p=0.089), with male predominance (51.7%vs63.5%;p=0.272), mostly located in the left colon (55,2%vs44.8%;p=0.314) and number and mean size of 1.46±0.88 and 6.89±6.56mm, respectively. After multivariate analysis, colorectal polyps were associated with F4 (34.5%vs14.9%;p=0.026;OR=3.01) and obesity (BMI>30Kg/m^2:55.2%vs29,7%;p=0.016;OR=2.91); hyperplastic polyps were associated with fibrosis for a cut-off of 6.9Kpa (AUROC:0.689;p=0.008;S=85.7%;Sp=51.2%), mainly F4 fibrosis (OR=4.38;p=0.004), hyperuricemia/gout (OR=3.35;p=0.042) and peptic ulcer disease (OR=8.53;p=0.043); adenoma was associated with liver steatosis (OR=3.50;p=0.024), F4 (OR=3.24;p=0.041) and obesity (OR=2.96;p=0.040); advanced adenoma/adenocarcinoma was associated with F4 (OR=1.224;p=0.021), hyperuricemia/gout (OR=1,50;p=0.044) and dilated cardiomyopathy (OR=1.24;p=0.003).

Conclusions: More than 1/4 of patients with NAFLD have colorectal polyps, being 16.5% adenoma and 4.8% advanced adenoma/adenocarcinoma. Obesity and liver steatosis are independent risk factors for colorectal adenoma. Liver fibrosis, especially F4 is an independent risk factor for all types of colorectal polyps.

Disclosure of Interest: None Declared

P06-03YI

Re-defining the Alanine aminotransferase upper limit of normal improves the prediction of metabolic syndrome risk

Martin Janičko*[1], Peter Jarcuska[1]

[1]1st. Department of Internal Medicine, Pavol Jozef Šafárik University in Košice, Faculty of Medicine, Košice, Slovakia

Corresponding author's email: martin.janicko@gmail.com

Introduction: Multiple studies have recently proposed the lowering of upper limit of normal (ULN) for alanine aminotransferase (ALT) to improve the diagnostic sensitivity for viral hepatitides and metabolix yndrome.

Aims: We have tried to validate some of the proposed ULNs in the diagnosis of metabolic syndrome (MS).

Material and Methods: We used data from HepaMeta Study conducted in 2011 in Slovakia which explored the prevalence of MS in the eastern Slovakia. Patients were tested for the criteria of metabolic syndrome and ALT. Different, previously published, ALT cutoffs were then used to calculate odds´ ratios, sensitivity, specificity and accuracy of MS and its components.

Results: Manufacturers´ recommended ULN used in our institution (0.8 ukat/L, 47 U/L for men and 0.6 ukat/L, 35 U/L for women) failed to predict any significant risk of MS. Lowered cut-off (72% of the original ULN) identified the patients with the highest age-adjusted risk of MS (OR 3.194, 95% CI 1.398-7.295). ALT was significantly associated with elevated levels of triacylglyceroles, hyperglycemia and obesity.

Conclusions: Decreased ULN of ALT is significantly associated with prevalence of metabolic syndrome. There is the need for discussion about the feasibility of lower ALT ULN in the clinical praxis.

Disclosure of Interest: None Declared

P06-04

The role of RANK and RANKL in the progression of NAFLD

Mengyue Hu[*1,2], Franck Phan[1,2], Isabelle Hainault[1,2], Vlad Ratziu[1], Olivier Bourron[1,2], Fabienne Foufelle[1,2]

[1]University Pierre and Marie Curie, [2]Group Cellular and Clinical Pathogenesis of Type 2 Diabetes, INSERM UMRS1138, Cordeliers Research Centre, Paris, France

Corresponding author's email: mengyuehu@gmail.com

Introduction: Chronic inflammation in liver plays an important role in the development of non-alcoholic fatty liver disease (NAFLD), a metabolic disease that encompasses a wide spectrum of diseases. The activation of nuclear factor-κB (NF-κB) in liver contributes to insulin resistance, steatosis, hepatic inflammation and fibrosis. Among the numerous systems that activate NF-κB, RANK and its ligand RANKL have been reported to be involved in the progression of insulin resistance and other diseases.

Aims: The aim of the project is to study the RANK/RANKL/NF-κB pathway in the onset and progression of NAFLD.

Material and Methods: The expression of RANK and RANKL in liver was measured, from NAFLD patients and NAFLD-related animal models. To evaluate which cell types is more sensitive upon RANKL activation, different cells including bone marrow derived macrophages (BMDMs) and primary hepatocytes were isolated ex vivo and challenged with recombinant RANKL. To study the gain of function of RANK receptor on lipid metabolism, mice overexpressing RANK receptor in myeloid cells were studied under normal chow (NC) or methione and choline deficient diet (MCD).

Results: The expression of RANK was markedly induced in liver, from both NASH patients and NASH-related rodent models, and the induction became more remarkable as the inflammatory states aggravated. Compared to hepatocytes, resident macrophages may play a more important role in RANK and RANKL signaling. On one hand, RANK expression was much higher in Kupffer cells compared to hepatocytes. On the other hand, upon in vitro RANKL stimulation, pro-inflammatory cytokine expressions were increased in macrophages, rather than in hepatocytes. Mice overexpressing RANK in myeloid cells displayed increased hepatic lipogenesis, which might source from the increased insulin sensitivity. In addition, these RANK-overexpressing mice also showed induced hepatic inflammation and a higher risk of developing steatohepatitis when challenged with MCD diet.

Conclusions: The expression of RANK was increased in liver under conditions related to NAFLD. The activation of RANK/RANKL pathway in macrophages could play a role in the lipid metabolism of liver. Detailed mechanism remains unclear.

Disclosure of Interest: None Declared

P06-05YI

miR-29a*, a potential microregulator of lipid droplet formation in the liver

Merna H. Hamad[*] [1], Noha M. Elemam[2], Shereen A. Elsobky [3], Injie O. Fawzy [4], Ahmed I. Abdelaziz [5]

[1]Pharmaceutical Biology Department, Faculty of Pharmacy and Biotechnology, German University in Cairo, Cairo, Egypt, [2]Sharjah Institute for Medical Research (SIMR), College of Medicine, University of Sharjah, Sharjah, United Arab Emirates, [3]Pharmaceutical Biology Department, Faculty of Pharmacy and Biotechnology, [4]Pharmacology and Toxicology Department, Faculty of Pharmacy and Biotechnology, German University in Cairo (GUC), Cairo, [5]School of Medicine, NewGiza University (NGU), Giza, Egypt

Corresponding author's email: merna-hatem9@hotmail.com

Introduction: Abnormal hepatic lipid regulation can lead to excess accumulation of intracellular fats, thus contributing to non-alcoholic fatty liver disease (NAFLD). Low-density lipoprotein receptor (LDLR) has been identified as a cell surface receptor responsible for lipid uptake and homeostasis.

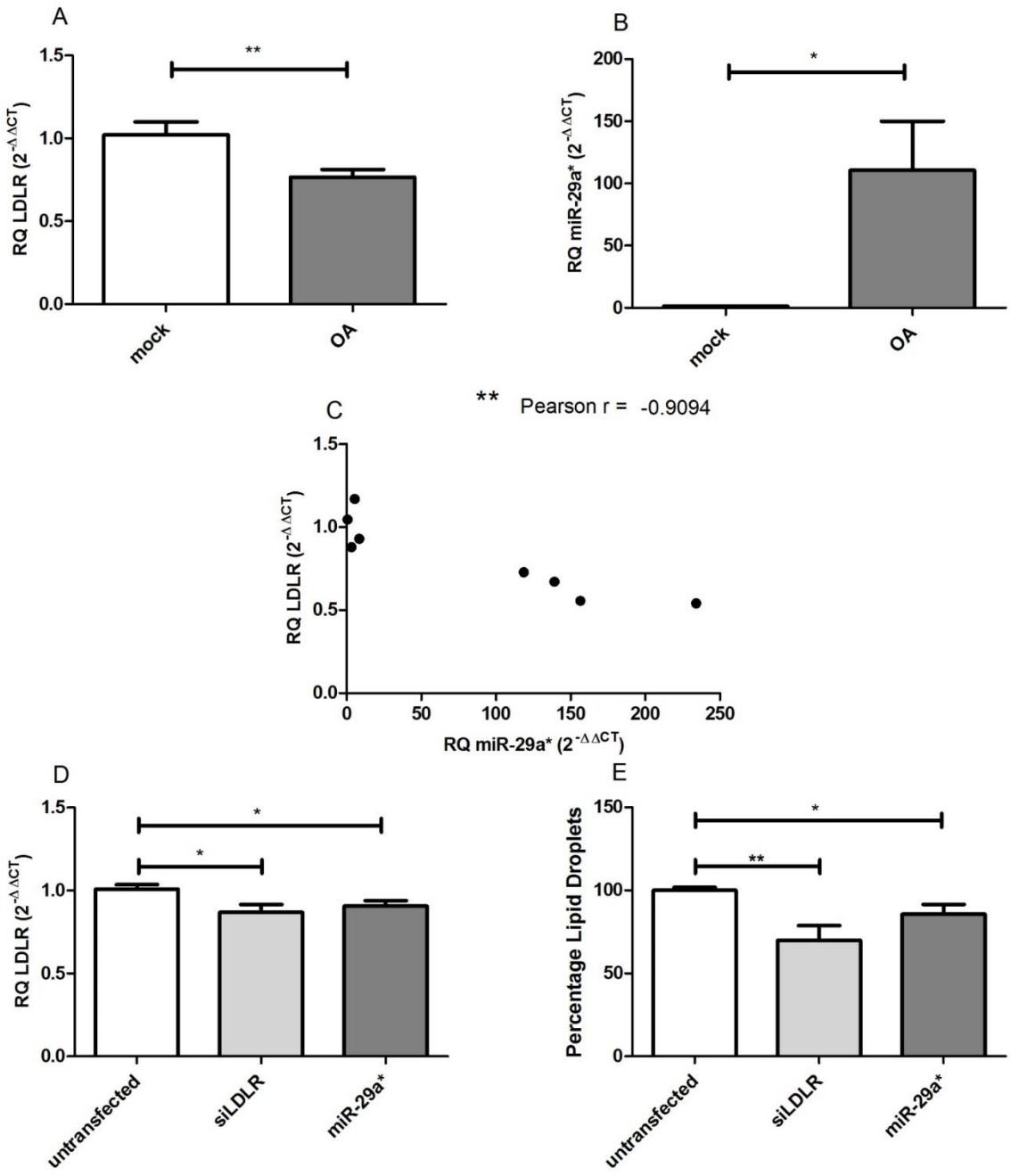
Aims: This study aimed at investigating the contribution of LDLR in the development of steatosis and identifying the regulatory role of selected microRNAs (miRNA or miR) on LDLR in an attempt to understand their involvement in lipid droplet (LD) formation in the liver.

Material and Methods: Huh-7 cells were treated with oleic acid (OA) to induce LD formation. Bioinformatics software were used to select miRNAs with the potential to regulate LDLR. miRNA and LDLR mRNA expression levels were assessed in transfected and untransfected OA-treated Huh-7 cells using qRT-PCR; moreover, the intracellular LD content was assessed by staining LDs using oil red-O dye then quantifying the amount of staining spectrophotometrically.

Results: OA-treated Huh-7 cells showed a significant downregulation of LDLR expression compared to untreated Huh-7 cells. miR-29a* was selected as a potential regulator of LDLR using *in silico* analysis. miR-29a* expression was increased in OA-treated cells, with a significant negative correlation observed between LDLR mRNA and miR-29a* relative expressions. Next, the effect of manipulating the intracellular levels of miR-29a* and its effect on LDLR mRNA expression was evaluated. Silencing of LDLR with siRNAs and transfection with miR-29a* mimics both led to a significant downregulation of LDLR mRNA levels. Finally, the impact of altering LDLR levels on the intracellular LD content was assessed. Both knockdown of LDLR with siRNAs and transfection of miR-29a* mimics were shown to significantly reduce intracellular LD content.

Conclusions: This study highlights a novel role of miR-29a* in modulating the gene expression of LDLR as well as regulating hepatic LD content, and offers novel insights into the pathogenesis of NAFLD.

Figure:



Disclosure of Interest: None Declared

P06-06

Liver-specific biomarker discovery using a diet-induced obese and biopsy-confirmed mouse model of non-alcoholic steatohepatitis

Michael Feigh[*1], Jane Palsgaard[1], Kristoffer Rigbolt[1], Maria B. Kristiansen[1], Sanne S. Veidal[1], Niels Vrang[1], Jacob Jelsing[1]

[1]GUBRA, Hørsholm, Denmark

Corresponding author's email: mfe@gubra.dk

Introduction: Non-Alcoholic SteatoHepatitis (NASH) is being diagnosis based on an invasive liver biopsy with a risk of complications. In addition, evaluation of primary endpoints in clinical trials for NASH are based on biopsy qualitative histopathological scorings with inherent marked intra-and interobserver variability. Hence, there is an unmet need for validated and robust hepatopathology-specific biomarkers for unbiased and accurate determination of disease severity and stage, which will also improve the basis for determining therapeutic efficacy in NASH.

Material and Methods: Male C57Bl6/J mice were fed a diet high in fat to induce diet-induced obesity (DIO) or high in trans-fat, fructose and cholesterol to induce NASH (DIO-NASH). Disease progression was determined by performing a liver biopsy. The therapeutic effect of dietary (chow) vs. pharmacological intervention with compounds in advanced clinical trials for NASH (liraglutide, obeticholic acid (OCA), elafibranor) was investigated in DIO-NASH. RNA sequencing (RNAseq) was performed on liver samples and identified gene transcriptional regulations in DIO-NASH mice wereas filtered against corresponding RNAseq data sets in DIO mice.

Results: DIO animals had pronounced changes in hepatic lipid metabolic pathways but did not show the upregulation in inflammatory and fibrotic gene components strongly manifested in DIO-NASH animals. RNAseq data from DIO-NASH vs. DIO mice biomarker candidates were identified based on gene regulation, expression level, and feasibility as a biomarker (*e.g.,* predicted secreted molecule). Several known biomarker candidates were identified by this approach (including vimentin and cytokeratin-18), indicating methodological proof of concept. A panel of novel biomarker candidates for NASH pathology was collected. Notably, dietary and pharmacological interventional strategies elicited highly different effects on these biomarkers.

Conclusions: Using a DIO-NASH mouse model of biopsy-confirmed NASH, we identified a panel of novel biomarker candidates for NASH. We demonstrate that disease interventional strategies modulate the regulation of these biomarker candidates. Clinical validation of the biomarker panel in human blood samples from different NAFLD patient populations is in progress, with the aim to select biomarker sets that may be instrumental for patient stratification in terms of disease stage, prognosis and treatment intervention strategy in NASH.

Disclosure of Interest: M. Feigh: Employee: Gubra, J. Palsgaard: Employee: Gubra, K. Rigbolt: Employee: Gubra, M. Kristiansen: Employee: Gubra, S. Veidal: Employee: Gubra, N. Vrang: Other: Gubra Founder, J. Jelsing: Other: Gubra Founder

P06-07

Reversal of liver fibrotic processes with XT-556, a stroma-activated macromolecular drug conjugate

Michail Shipitsin[*] [1], Jenny Liu[1], Sung Jin Huh[1], Kyriakos Economides[1], Bhava Vangamudi[1], Allison Neenan[1], James Ackley[1], Joelle Baddour[1], William Dahlberg[1], Sattanathan Paramasivan[1], Paul Kopesky[1], Donald Chickering[1], Matthew Golder[2], Farrukh Vohidov[2], Jeremiah Johnson[2], Jannik Andersen[1], Peter Blume-Jensen[1]

[1]Xtuit Pharmaceuticals, Waltham, [2]Chemistry, Massachusetts Institute of Technology, Cambridge, United States

Corresponding author's email: mshipitsin@xtuit.com

Introduction: The Renin-angiotensin system (RAS) is an important regulator of liver inflammation and fibrosis development. Angiotensin receptor blockers (ARBs) lower RAS activity through inhibition of the angiotensin 2 type 1 receptor but their application to treatment of liver disease is hampered by systemic effects, including blood pressure lowering. Using a novel chemical approach we designed a macromolecule pro-drug (XT-556) version of the ARB telmisartan for preferential delivery and release of active drug in the diseased liver.

Aims: We evaluated XT-556 for anti-fibrotic efficacy in a mouse CCL4-induced liver fibrosis model. We assessed tissue biodistribution by both PK profiling of released and conjugated telmisartan and by whole organ imaging. We tested safety and toxicity in mice, rats, and dogs.

Results: In healthy mice, after IV administration of Cy7.5-labeled XT-556, there was preferential accumulation of fluorescence in liver and spleen, consistent with PK measurements. Mass-spectrometry based measurements demonstrated a >100-fold higher concentration of released Tel in the liver compared to blood and a steady-state drug release in liver tissue even 15 days after administration. In contrast to telmisartan, XT-556 had no effect on blood pressure in mice or dogs even after repeated dosing at maximal feasible dosage. Histopathological evaluation of multiple organs showed no signs of toxicity after repeated dosing in mice, rats and dogs. We tested XT-556 in a CCL4-induced mouse liver fibrosis model. Treatment with XT-556 unlike telmisartan dramatically reduced plasma levels of ALT and AST and bilirubin. XT-556 had a periportal hepatocyte necrosis protective effect, with decreased pathology necrosis scores and inhibition of liver cell apoptosis together with restored liver glycogen content. XT-556 treatment significantly downregulated stromal and stellate cell activation state markers, e.g. Nestin and levels of active Stat3, a downstream AT1R mediator. Consistently, a global gene expression analysis revealed a strong downregulation of pro-fibrotic and inflammatory genes in response to XT-556 treatment.

Conclusions: XT-556 is a novel macromolecule drug conjugate that enables superior delivery and long term release of telmisartan into fibrotic liver tissue with lower systemic levels avoiding blood pressure drop. The improved therapeutic index and great tolerability of XT-556 in animals warrants further development towards treatment of human fibrotic liver disorders.

Disclosure of Interest: M. Shipitsin: Employee: Xtuit Pharmaceuticals, J. Liu: Employee: Xtuit Pharmaceuticals, S. J. Huh: Employee: Xtuit Pharmaceuticals, K. Economides: Employee: Xtuit Pharmaceuticals, B. Vangamudi: Employee: Xtuit Pharmaceuticals, A. Neenan: Employee: Xtuit

Pharmaceuticals, J. Ackley: Employee: Xtuit Pharmaceuticals, J. Baddour: Employee: Xtuit Pharmaceuticals, W. Dahlberg: Employee: Xtuit Pharmaceuticals, S. Paramasivan: Employee: Xtuit Pharmaceuticals, P. Kopesky: Employee: Xtuit Pharmaceuticals, D. Chickering: Employee: Xtuit Pharmaceuticals, M. Golder: Other: Xtuit Pharmaceuticals, F. Vohidov: Other: Xtuit Pharmaceuticals, J. Johnson: Other: Xtuit Pharmaceuticals, J. Andersen: Employee: Xtuit Pharmaceuticals, P. Blume-Jensen: Employee: Xtuit Pharmaceuticals

P06-08YI

Noninvasive assessment of fibrosis in NAFLD patients using platelet count and platelet indices

Milica Stojkovic Lalosevic[*] [1], Nevena Jocic[2], Igor Dumic[3, 4], Mirjana Stojkovic[2], Tamara Alempijevic[2], Aleksandra Pavlovic Markovic[2]

[1]Hepatology, [2]Clinical center of Serbia, Belgrade, Republic of Serbia, [3]Mayo Clinic College of Medicine, Rochester, MN, [4]Mayo Clinic Health System, Eau Claire, WI, United States

Corresponding author's email: drmilicastojkovic@gmail.com

Introduction: Bearing in mind NAFLD rising prevalence there is a need of non-invasive diagnostic markers for early detection and for monitoring disease progression.

Aims: The aim of our study was to determine whether platelets and MPV, PDW and PCT are efficient in predicting liver fibrosis as well as to compare the diagnostic accuracy of these indices in predicting liver fibrosis with other known noninvasive fibrosis scores.

Material and Methods: All patients were diagnosed with NAFLD based on prior interview, clinical features, physical examination, laboratory tests and ultrasound imaging. Analysis of hematological parameters along with platelets and their indices were performed in whole blood anticoagulated with EDTA within 4 hours after collection. Hematological as well as other biochemical parameters of NAFLD patients were compared with healthy individuals.

Results: NAFLD patients had lower PC and higher MPV, PCT, PDW compared to controls ($P<0.05$). When we stratified NAFLD patients based on presence of fibrosis, we noticed there was statistically significant difference in average values of PDW and PC between groups ($P<0.05$). We found statistically significant negative correlation of PC and APRI ($P=0.00$, $r=-0.530$), FIB-4 ($P=0.00$, $r=-0.480$) and NFSP=0.00, $r=-0.320$) scores respectively. Also we have found statistically significant negative correlations of PDW and APRI ($P=0.00$, $r=-0.5629$), FIB-4 ($P=0.00$, $r=-0.553$) and NAFLD ($P=0.00$, $r=-0.346$) scores respectively.

Conclusions: Patients with NAFLD have significant increase in values of PCT, PDW and MPV. There is a need for future studies to investigate potential use of these test in diagnostic algorithm of NAFLD patients.

Disclosure of Interest: None Declared

P06-09

Impact of treating hyperuricemic NAFLD patients with Allopurinol on cytokeratin 18: A pilot study

Mohamed A. Mokhles*[1] on behalf of Osama Badary , Salwa Salama , Yaser El Hossary , Sylvia Samir , Osama Badary[2], Salawa Salama[3], Yasser El Hossary[1], Sylvia Fouad[3]

[1]General Medicine, National Research Center, [2]Clinical pharmacy, Faculty of pharmacy, [3]Pharmacology, National Research Center, Cairo, Egypt

Corresponding author's email: mmokhlesoct73@yahoo.com

Introduction: Elevated Uric acid (UA) strongly associates non alcoholic fatty liver disease (NAFLD) , in the same time there is a growing body of evidence pointing to the role of cytokeratin (CK) 18 as NAFLD serological marker

Aims: to evaluate the impact of the UA lowering drug Allopurinol in treatment of hyperuricemic NAFLD patients on CK18.

Material and Methods: 31 hyperuricemic ultrasound (US) NAFLD diagnosed patients of a mean age of 44.3 ± 11 years 32% females were enrolled into the study and grouped into; Group A (14 patients): who received starch based tablets and Group B (17 patients): who received allopurinol (100-300 mg). The study was carried on for 3 months for both groups. UA, cytokeratin (CK) 18, adiponectin, and fatty liver (FL) grade by US were measured at baseline and at the end of the study.

Results: The study showed a significant decline in group B as regards; CK18 (p=0.006), yet, neither of the 2 groups showed change in adiponectin or FL grade by US, while UA declined in both groups A and B (p=0.05 and p<0.001 respectively)

Conclusions: Allopurinol treatment for hyperurecemic NAFLD patients may stop the disease progression as mirrored by CK18 decline, which warrants further studies on a large scale.

Disclosure of Interest: None Declared

P06-10

Monocyte chemoattractant protein-induced protein 1 regulates PPARs in hepatic steatosis

Natalia Pydyn*[1], Ewelina Pośpiech[2], Jolanta Jura[1], Jerzy Kotlinowski[1]

[1]Department of General Biochemistry, [2]Malopolska Centre of Biotechnology, Jagiellonian University, Kraków, Poland

Corresponding author's email: natalia.pydyn@doctoral.uj.edu.pl

Introduction: Monocyte chemoattractant protein-induced protein 1 (MCPIP1) is an endoribonuclease, that degrades selected mRNAs, miRNAs and viral RNAs. Through degradation of proinflammatory cytokines transcripts, MCPIP1 functions as a negative regulator of inflammation. It was also shown than MCPIP1 inhibits adipogenesis, by direct cleavage of C/EBPβ mRNA. The main feature of non-alcoholic fatty liver disease (NAFLD) is excessive accumulation of lipid droplets in hepatocytes, which next can be followed by development of inflammation.

Aims: Since MCPIP1 is a negative regulator of inflammation and plays important role in lipid metabolism, our aim was to test its function in NAFLD.

Material and Methods: Experiments *in vitro* were performed using HepG2 cell line stimulated with sodium oleate. To assess role of MCPIP1 in cellular model of steatosis, we compared cells overexpressing MCPIP1 under control of tetracycline-inducible promoter, cells transduced with vector coding for shMCPIP1 sequence and control cells. RNA samples isolated from cells overexpressing MCPIP1 and control cells were subjected to high-throughput sequencing provided by Ion Ampliseq™ technology and Ion Proton™ system. Activity of PPARs was measured by luciferase reporter assay and ELISA.

Results: Our results showed that stimulation of HepG2 cells with sodium oleate induces MCPIP1 expression in a time and dose dependent manner. We have found that MCPIP1 overexpression in cells treated with oleate enhanced lipid accumulation and induced level and activity of peroxisome proliferator-activated receptors (PPARs). MCPIP1 overexpression also changed mRNA profile in comparison to control cells, among 99 genes induced by MCPIP1 we detected induction of PPARα (e.g. VNN1) and PPARγ (e.g. CD36) targets. Moreover, MCPIP1 led to decreased expression of genes related to lipid export (e.g. MTTP).

Conclusions: Obtained results indicate important role of MCPIP1 in the context of NAFLD. By regulation of PPARs level and activity, MCPIP1 has an impact on lipid metabolism in hepatocytes.

Disclosure of Interest: None Declared

P07-01

Low TLR9 expression in adaptive cells protects against progression of nonalcoholic fatty liver diseases

Nadia S. Alegre[1], Cecilia C. Garcia[1], Luis A. Billordo[1], Javier Benavides[* 2], Luis Colombato[2], Daniel Poncino[3], Daniel García[3], Alejandra C. Cherñavsky[1]

[1]Gastrointestinal immunology, INIGEM Institute, [2]Hepatology, Hospital Británico, [3]Hepatology, Sanatorio Mendez ObSBA, CABA, Argentina

Corresponding author's email: alejandra.cher@yahoo.com.ar

Introduction: T cells play a key role on the progress of nonalcoholic fatty liver diseases (NAFLD). Signaling through toll-like receptors (TLR) co-stimulates the activation, differentiation and generation of memory T cells. No study correlates clinical facts in NAFLD with TLR9 expression and function of adaptive cells.

Aims: To study the potential link between metabolic changes and TLR9 expression in peripheral and hepatic adaptive T cells and on the co-stimulatory activity and generation of peripheral memory T cells

Material and Methods: Blood and liver samples came from 25 patients with simple steatosis (SS), 34 steatohepatitis (NASH) and 81 controls (Co). Body mass index, waist circumference, total serum triglyceride, cholesterol (mg/dl), fasting blood glucose (mg/dL), serum alanine and aspartic aminotransferase activities (ALT/AST) (IU/l) were measured. From peripheral blood mononuclear cells (PBMC), $CD3^+$ cells were negatively selected using immunobeads and stimulated with anti-CD3 (250 ng/mL) +/- CpG-OdN (2 mM). Cell suspensions were obtained by mechanical treatment of liver biopsies. Cells were stained with anti-CD4, -CD8, -TLR9, -CD69 or -IFNg mAbs and studied by flow cytometry. The frequency of $CCR7^-$ cells was calculated as a ratio within CD4+ or CD8+ CD45RO cells. TLR9 expression was calculated as shown in the index: [TLR9 expression $_{CCR7-}$ / TLR9 expression $_{CCR7^+ + CCR7^-}$]. Mann-Whitney, Kruskal-Wallis and Spearman´s correlation tests were used.

Results: Within patients with NAFLD, we found a correlation between plasma levels of AST and ALT and TLR9 expression in peripheral $CD8^+$ cells (r=0.645, p=0.037; r=0.645, p=0.034) and also between plasma triglyceride levels and TLR9 expression in hepatic $CD4^+$ cells (r=0.821; p=0.034). Patients with SS showed a low TLR9 expression ($CD4^+$: p=0.022, $CD8^+$: p=0.002; vs. Co) and a lower frequency of differentiated $CD8^+$ $IFNg^+$-producing cells (p=0.002, vs. NASH) in periphery, together with a decreased expression of TLR9 (CD4: p=0.020; vs. Co) in liver. A higher frequency of peripheral $CD4^+CCR7^-$ cells (p=0.013, vs. Co) was found but unrelated to a higher expression of TLR9 in $CCR7^-$ cells.

Conclusions: A global decrease of TLR9 expression in adaptive cells is linked to distinctive metabolic changes associated with NAFLD. In addition, the relation described between T cell activation and TLR9 expression evidences its protective role against an increased differentiation of $CD8^+$ cells at early stages of NAFLD progression.

Disclosure of Interest: None Declared

P07-02YI

Q Liver: a novel automated system for assessment of liver steatosis and fibrosis in routine histological images from patients with NAFLD

Roberta Forlano*[1], Benjamin H. Mullish[1], Nikolaos Giannakeas[2], Alexandros Tzallas[2], Markos Tsipouras[3], Michael Yee[1], Simon Taylor-Robinson[1], Jo Lloyd[4], Robert Goldin[4], Mark Thursz[1], Pinelopi Manousou[1]

[1]Department of Hepatology, Imperial College, London, United Kingdom, [2]Departmenf of Computer Engineering, Technological Educational Institute, Epirus, [3]Department of Informatics and Telecommunication Engineering, Greece, Greece, Greece, [4]Department for Cellular Pathology, Imperial College, London, United Kingdom

Corresponding author's email: robertaforl@gmail.com

Introduction: Liver biopsy is the reference standard for diagnosing/ staging NAFLD. However, steatosis grade and fibrosis stage are currently reported using semi-quantitative scores, with significant associated inter-and intra-observer variability.

Aims: We aimed to develop an automated method to objectively quantitate steatosis and fibrosis using NAFLD patients' biopsies. We compared these readings to Liver Stiffness Measurements (LSM) and controlled attenuation parameter (CAP) scores.

Material and Methods: 246 consecutive patients with biopsy-confirmed NAFLD and transient elastography within 3 months of biopsy were evaluated. Biopsies were independently scored by two histopathologists and digitalised at 2x magnification. Areas of steatosis and fibrosis were annotated manually using the NDP.view2 to facilitate machine-learning. Each image was then analysed by the automated software: computed fat percentage (fat%) and Collagen Proportionate Area (CPA) were compared with manual annotation, and were also correlated with LSM/ CAP.

Results: There was excellent concordance between manual and automatic measurements (interclass correlation coefficient, ICC=0.98, (95%CI=0.96-0.99, p=0.0001)). There was good correlation between fat% and steatosis grade, but significant overlap between groups. Results were similar between CPA and fibrosis stage. LSM associated significantly with CPA (Rho=0.8, p=0.001). CAP score correlated significantly with fat% (Rho=0.45, p=0.002) and effectively diagnosed steatosis>5% (AUROC 0.82, 95% sensitivity, 60% specificity), but could not distinguish between grades.

Conclusions: Automated software, using low-resolution images, can provide rapid, objective assessment of steatosis and fibrosis in NAFLD, with excellent correlation with experts' annotation. This tool may aid objective assessment of therapeutic response in clinical practice and in trials for patients with NAFLD.

Disclosure of Interest: None Declared

P07-03YI

Possibilities of non-invasive assessment of the formation and progression of liver fibrosis in patients with non-alcoholic fatty disease

Saidrakhim N. Lukmonov* [1]

[1]*faculty surgery, Tashkent Medical Academy, Tashkent, Uzbekistan*

Corresponding author's email: saidrakhim.lukmonov@mail.ru

Aims: To assess the diagnostic significance and informativeness of clinical and laboratory markers for the formation and progression of liver fibrosis in non-alcoholic fatty liver disease (NAFLD).

Material and Methods: In the open case-control study, we examined 77 patients with NAFLD on the basis of faculty surgery at the Tashkent Medical Academy. The analysis of the anamnesis of life and disease, anthropometric indicators, biochemical blood test, ultrasound of the abdominal, insulin levels, leptin, adiponectin, matrix metalloproteinases (MMP-9) and their inhibitors (TIMP-1 and 2), liver elastometry. Depending on the stage of fibrosis (0-3), the subjects were divided into 4 groups: n = 33, n = 22, n = 17, n = 5, respectively.

Results: NAFLD in the stage of steatosis was diagnosed in 42 (54.5%) patients, steatohepatitis - 35 and 45.5%, respectively. Statistically significantly more often, the stage of steatohepatitis was noted in patients with stage 3 fibrosis (2I = 9.86, p <0.05). Patients with severe fibrosis had higher values of systolic blood pressure (SAP) (H = 9,426, p <0,02) and waist-to-hip ratio (H / 8,706, p <0,03). In individuals with fibrosis of stage 1, the liver was enlarged more often in palpation studies (2I = 10.11, p <0.05). As the fibrosis stage increased, the levels of OT / OB (p <0.03), ALT / AST (p <0.04), TIMP 2 (p <0.04) increased statistically significantly. Dimensions of the liver (J = 66.7, 16.0, 104.8 for fibrosis 1, 2, 3 stages, respectively), glucose level (J = 0.1, 22.5, 54.5), SAP (J = 40.3, 3.8, 21.2), LDL (J = 14.7, 73.4, 73.4), OT / OB (J = 57.0, 26.0, 159.0), ALT / AST (J = 1.2, 20.5, 116.1) and MMP 9 levels (J = 5.1, 2.4, 59.4), TIMP 1 (J = 33.7, 14.7, 6.6), TIMP 2 (J = 45.6, 12.3, 150.6) showed high information for diagnosis of various stages of liver fibrosis. At the same time, LDL, glucose, MMP 9 and leptin levels were the most informative in assessing the progression of the initial stages of fibrosis (stage 1 in 2), liver enlargement during examination, SAP level, presence of carbohydrate metabolism disorders, ALT / AST, OT / OB, 1 and 2 - with respect to the progression of fibrosis stage 2 to stage 3.

Conclusions: Clinical and laboratory indicators are highly informative for the diagnosis of fibrotic changes in the liver and are associated with the development of progressive stages of liver fibrosis in patients with NAFLD.

Disclosure of Interest: None Declared

P07-04

Development and characterization of a novel in vitro human liver fibrosis model for efficacy testing of anti-fibrotic drugs based on 3D human liver microtissues

Radina Kostadinova[1], Aparna Neelakandhan[1], Monika Kijanska[1], Natalia Zapiorkowska[1], Burcak Yesildag[1], Sabrina Steiert[1], Tobias Strassfeld[1], Patrick Guye[1], Simon Messner[*1]

[1]Insphero Ag, Schlieren, Switzerland

Corresponding author's email: simon.messner@insphero.com

Introduction: Liver fibrosis is the excessive accumulation of extracellular matrix proteins such as collagen, which can lead to cirrhosis. Anti-fibrotic therapies aim at inhibiting the accumulation of fibrotic cells and preventing the deposition of extracellular matrix proteins. The most frequently used model for *in vitro* liver fibrosis consist of simple mono-layer cultures of hepatic stellate cells (HSC), ignoring the role of hepatocyte injury or animal models of liver fibrosis, which are not predictive enough for the effect of the anti-fibrotic drugs in human. The novel model described herein incorporates all relevant cell types associated with liver fibrosis (hepatocytes, Kupffer cells, liver endothelial cells and HSC) and is therefore suggested to be a highly suitable tool for liver fibrosis research.

Aims: Development and characterization of a physiological relevant 3D Human Liver Fibrosis Model, amenable for anti-fibrotic drug screening.

Material and Methods: An *in vitro* human liver model was engineered to incorporate all the relevant liver cells such as hepatocytes, human HSC, Kupffer cells and liver endothelial cells. The resulting 3D InSight™ Human Liver Fibrosis model has been characterized under basal and induced liver fibrosis conditions on a morphological and phenotypic level by immuno-staining techniques and gene-expression analysis.

Results: Using the cell type specific markers and immunohistochemistry we demonstrated the presence of various cell types such as hepatocytes, HSC, Kupffer cells and endothelial cells during the cultivation and treatment period. TGFb-treatment activated the HSCs as detected by up to 8 fold increased expression of a-smooth muscle actin (a-SMA). In addition, increased expression of extracellular matrix gene expression such as collagen type I, III, IV and VI and the pro-fibrotic markers such as platelet-derived growth factor beta (PDGFb) and lysyl oxidase (Lox) was detected. Immunofluorescence staining showed an increase expression of a-SMA and Collagen type I/IV deposition in the TGFb-treated samples. Inhibition of fibrosis induction with anti-fibrotic drugs targeting ALK5 or tyrosine kinases effectively blocked fibrosis development.

Conclusions: We demonstrated that TGFb treatment induces liver fibrosis in vitro and that this model system allows studying efficacy of anti-fibrotic drugs. The 3D Human Liver Fibrosis model is thus a novel, biologically relevant *in vitro* model of liver fibrosis, suitable for high-throughput efficacy screening of anti-fibrotic drugs.

Disclosure of Interest: None Declared

P07-05

The role of decaffeinated coffee on gut permeability and lipid metabolism in a diet induced animal model of NAFLD

Vincenzo Lembo[*] [1], Giovanna Mazzone[1], Giuseppe D'Argenio[1], Paola Vitaglione[2], Antonella Rossi[1], Valentina Cossiga[1], Nicola Caporaso[1], Filomena Morisco[1]

[1]Department of Clinical Medicine and Surgery, Gastroenterology Unit, University of Naples Federico II, Naples, [2]Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy

Corresponding author's email: v.lembo@hotmail.it

Introduction: In recent years an increasing number of evidence linking the alteration of gut permeability to Non Alcoholic Fatty Liver Disease (NAFLD) are available.

Aims: The aim of this study was to evaluate the protective effect of decaffeinated coffee on NAFLD evaluating the structure of the tight junction and the intestinal permeability in a mice model of NAFLD induced by high-fat diet.

Material and Methods: C57BL/6 mice were divided into 3 groups (n=8 per group). First group was fed with standard diet (SD, 3.3 Kcal/g, 5% from fat,), second group was fed with high fat diet (HFD, 5.6 Kcal/g, 58% from fat), third group was fed with HFD and decaffeinated coffee solution (HFD+Coffee) for 12 weeks. The decaffeinated coffee powder was diluted in water to afford mice a daily dosage of coffee corresponding to a daily dose of 6 cups of espresso coffee or 2 cups of filtered coffee for a 70 kg person.

Results: After 12 weeks, HFD and HFD+Coffee mice had higher energy intakes than SD mice (p=0.0001 and p<0.0001, respectively). HFD and HFD+Coffee mice weighed significantly more than SD mice (p=0.0003 and p=0.0008, respectively). Body weight was significantly lower in HFD+Coffee group *vs* HFD group (p=0.0033). Notably, the differences in body weight between HFD group and HFD+Coffee group progressively increased during the study period and became significant at week 8 (p=0.028). Coffee supplementation significantly reversed the HFD–induced increase of serum cholesterol (p<0.001) and ALT (p<0.05), but had no effect on triglycerides. Furthermore, coffee supplementation significantly ameliorated liver macrovesicular steatosis (p<0.001) and ballooning degeneration (p<0.05). HFD+Coffee mice showed increased expression of the duodenal and colonic Zonulin (ZO)-1 as well as duodenal claudin compared to HFD mice (p<0.05).

Conclusions: Data show that coffee supplementation reduced body weight gain in HFD mice and reverse the status of NAFLD by reducing serum cholesterol, ALT, macrovesicular steatosis and ballooning degeneration. Furthermore, coffee supplementation reduces gut permeability by increasing the ZO-1 and claudin expression.

Disclosure of Interest: None Declared

P07-06

The effect of PNPLA3, NCAN, TM6SF2 and MBOAT7 variants on hepatocellular carcinoma occurrence and prognosis in relation to underlying etiologies of liver disease

Natthaya Chuaypen[1], Maneerat Raksayot[1], Nutcha Pinjaroen[2], Pisit Tangkijvanich[*] [1] and Research Unit of Hepatitis and Liver Cancer

[1]Department of Biochemistry, Faculty of Medicine,Chulalongkorn University, [2]Radiology, Faculty of Medicine,Chulalongkorn University, Bangkok, Thailand

Corresponding author's email: pisittkvn@yahoo.com

Introduction: Single nucleotide polymorphisms (SNPs) in the patatin-like phospholipase domain containing 3 (*PNPLA3*), neurocan (*NCAN*), transmembrane 6 superfamily 2 (*TM6SF2*) and membrane-bound O-acyltransferase domain-containing protein 7 (*MBOAT7*) are associated with fatty liver disease. It is not clear whether they could be attributed to the occurrence and prognosis of hepatocellular carcinoma (HCC).

Aims: This study was aimed at evaluating the association between these SNPs and HCC in Thai patients with different etiologies of liver disease.

Material and Methods: The *PNPLA3* (rs738409), *NCAN* (rs2228603), *TM6SF2* (rs58542926) and *MBOAT7* (rs641738) variants were determined by allelic discrimination in blood samples of 105 healthy controls and 415 patients with HCC [205 HBV-related HCC (HBV-HCC), 109 HCV-related HCC (HCV-HCC), and 101 non-B non-C-related HCC (NBNC-HCC)].

Results: Mean age and gender distribution were comparable between groups of HCC. NBNC-HCC group had significantly higher prevalence of diabetes mellitus and heavy drinking behavior than HBV-HCC and HCV-HCC groups (P<0.001). The frequency of G allele of *PNPLA3* rs738409 variant was significantly higher in NBNC-HCC (47%) compared to those in healthy controls (32%), HBV-HCC (34%) and HCV-HCC (32%) (P=0.026). Similarly, T allele of *TM6SF2* rs58542926 was more prevalent in NBNC-HCC (43%) than in healthy controls (8%), HBV-HCC (10%) and HCV-HCC (11%) (P=0.001). The distributions of remaining SNPs were not significantly different between groups. In logistic regression analysis, *PNPLA3* rs738409 (OR=1.79, 95%CI: 1.09–2.93; P=0.021) and *TM6SF2* rs58542926 (OR=2.13, 95%CI: 1.29–3.52; P=0.003) were independently associated with NBNC-HCC compared to viral-related HCC after adjustment for the effects of age, gender and body mass index. In addition, the proportion of patients with NBNC-HCC increased significantly along with the increase of the number of risk alleles of *PNPLA3* rs738409 and *TM6SF2* rs58542926. There were no association between these SNPs and overall survival of patients with HCC.
Conclusions: The G allele of *PNPLA3* rs738409 and T allele of *TM6SF2* rs58542926 were more frequently distributed among patients with NBNC-HCC but not among patients with viral-related HCC. Thus, these risk alleles might influent the occurrence of HCC in patients with NBNC-HCC. However, all the studied SNPs were not associated with the prognosis of HCC.

Disclosure of Interest: None Declared

P07-07YI

Probiotics and nutraceuticals: are they one team players in NAFLD prevention

Nazarii Kobyliak[1], Natalia Bosak[*] [1], Tetyana Falalyeyeva[2], Tetyana Beregova[2], Lyudmyla Ostapchenko[2]

[1]Bogomolets National Medical University, [2]Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

Corresponding author's email: nazariikobyliak@gmail.com

Introduction: Today probiotics have been suggested as a treatment for the prevention of non-alcoholic fatty liver disease (NAFLD). Omega-3 fatty acid treatment may have beneficial effects in regulating hepatic lipid metabolism, adipose tissue function, and inflammation. Smectite is a natural silicate that has the ability to bind endo- and exotoxins and restored the barrier properties of human intestinal cell monolayers.

Aims: The study aims to determine whether probiotics plus nutraceuticals such as smectite or omega-3 are superior to probiotic alone on the monosodium glutamate (MSG) induced NAFLD model in rats.

Material and Methods: Totally 75 rats divided into 5 groups were included (n=15, in each). Rats of group I were intact. Newborns rats of groups II-IV were injected with MSG. The III (Symbiter) group received 2.5 ml/kg of multiprobiotic "Symbiter" containing concentrated biomass of 14 probiotic bacteria genera. The IV (Symbiter-Omega) and V (Symbiter+Smectite) groups received combination of probiotic biomass supplemented with flax and wheat germ oil (250 mg of each, concentration of omega-3 fatty acids 1-5%) or smectite gel (250 mg) respectively. To assess morphological changes in liver we used NAS (NAFLD activity score).

Results: In all interventional groups reduction of total NAS score was observed. Supplementation with omega 3 fatty acids lead to 20 % higher decreasing of steatosis score (0.73±0.11 vs 0.93±0.22, p=0.848) and reduction by 16.6 % of triglycerides content in liver as compared to probiotic alone. Co-treatment with Symbiter+Smectite are associated with more pronounced reduction of lobular inflammation (0.13±0.09 vs 0.33±0.15). Moreover, supplementation of probiotics with omega-3 lead to more pronounced decreasing of HOMA-IR (2.31±0.13 vs 4.02±0.33, p<0.001) and elevation of adiponectin level (5.67±0.39 vs 2.61±0.27, p<0.001) as compared to obesity group. Both nutraceuticals combination with probiotic and probiotic alone equally attenuated inflammation.

Conclusions: Our study demonstrated more pronounced reduction of steatosis and hepatic lipid accumulation after treatment with combination of alive probiotics and omega-3 as compared to probiotic alone. From the other hand, supplementation with smectite gel due to his absorbent activity and stabilization mucus layer properties can impact on synergistic enhancement of single effect which manifested with reduction of lobular inflammation and at list partly NASH prevention.

Disclosure of Interest: None Declared

P07-08

The severity of nonalcoholic fatty liver disease associated with insulin resistance and metabolic syndrome

Hojung Jung*[1], Yong Kyun Cho[1], Hyo Sun Lee[1], Sung Yun Lee[1], Jae Wan Lim[1]

[1]*Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea, South*

Corresponding author's email: hjh0610228.jung@samsung.com

Introduction: Hepatic fat accumulation have a key role in the pathogenesis of insulin resistance and metabolic syndrome in patients with NAFLD (nonalcoholic fatty liver disease), but the relationship of insulin resistance and metabolic syndrome between simple steatosis and NASH (nonalcoholic steatohepatitis) measured by MRI has not been established.

Material and Methods: A total of 609 middle-aged nondiabetic NAFLD subjects with or without metabolic syndrome [male n= 502 (82.4%), female n= 107 subjects (17.6%), median age, 51.8 yr; range, 31-79] quantified hepatic fat by using 1.5T MRI examination including double-echo chemical shift imaging for calculating fat fraction in the liver. Based on the presence or absence of steatosis on MRI and serum alanine aminotransferase (ALT), subjects were divided into control group, steatosis-alone group, and a group with presumed NASH with steatosis and elevated serum ALT(>40 IU/L). Using multinomial logistics regression analysis, we also measured the presence of insulin resistance and metabolic syndrome component of each group.

Results: The odds ratio for the presence of metabolic syndrome was 7.2 times higher in a NASH group (OR=7.23, 95%CI 4.23-12.34, P<0.001). Independent on both age and sex (OR=16.73, 95%CI 9.01-31.04, P<0.001), HOMA-IR (>2.5) was increased up to 16-fold over controls. Overall, insulin resistance and the presence of metabolic syndrome in NASH were significantly higher than those in either steatosis-alone group or control group. Among metabolic syndrome components, the increased waist circumference (>90cm, in male, >80cm, in female) was more strongly associated with a NASH group. (OR=5.96, 95%CI 3.56-9.97, P<0.001).

Conclusions: Insulin resistance and presence of metabolic syndrome including central obesity were key determinants in NASH patients. Our findings are useful to provide major targets to prevent the NASH development.

Disclosure of Interest: None Declared

P07-09YI

Discovery and validation of new modulators of necroptosis using phenotypic high throughput screening of large compound library

Hugo Brito[*] [1], Vanda Marques[1], Marta Afonso[1], Dean Brown[2], Ulf Börjesson[2], Nidhal Selmi[2], Martina Fitzek[2], Cecília Rodrigues[1]

[1]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisboa, Portugal, [2]AstraZeneca Pharmaceuticals, Discovery Sciences, Hit Identification, Cambridge, United Kingdom

Corresponding author's email: hugobrito1@gmail.com

Introduction: Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease in the Western world, affecting up to 30% of adult population and representing an important public health concern. Disruption of the equilibrium between cell death and proliferation may be at the origin of NAFLD pathogenesis. We have previously shown that regulated necrosis or necroptosis plays a key role in non-alcoholic steatohepatitis (NASH).

Aims: Here we aim to discovery novel, selective and potent inhibitors of necroptosis, which might evolve to potential therapeutic strategies. Benefiting from privileged research collaborations on target innovation between academia and pharma industry, we have gained access to a high-quality, large compound pharma collection of over 250,000 compounds.

Results: Compounds were screened at 30 µM for their ability to block TNF-α-induced necroptosis (30 µM; 8 h) in murine fibrosarcoma L929 cell line, using a bioluminescent cytolysis assay. From the full library screening, valid data was achieved for 251,879 compounds. For hit selection, exclusion criteria included qualitative and quantitative parameters, ZScore and percentage of cell death inhibition. A cut off threshold of > 30% inhibition of cell death by tested compounds and a ZScore < -10, led to 3,353 active hits, corresponding to 1.3% hit rate for the full library. For positive hits, dose-response curves were built using a 10-point concentration range of 0.004-100 µM to quantitatively assess inhibitory potency of selected compounds in murine L929 and human Jurkat T FADD(-/-) cell lines. Further selection comprised exclusion criteria of pEC50 < 5 (EC50 < 10 µM) in both cell lines, leading to 1,000 actives at 29.8% hit rate. Next, using Jurkat E6.1 cells stimulated with cycloheximide (0.5 µg/mL; 8 h) for apoptosis induction, selected compounds were tested in caspase-3/-7 enzymatic activity assays using a 4-point concentration range of 0.03-30 µM. Active hits protecting from apoptosis were excluded remaining 180 inactive compounds to be taken further. Moreover, 33 and 21 compounds showed RIPK1 and RIPK3 inhibitory kinase activity, respectively, although the vast majority protected from necroptosis through yet undetermined mechanisms of action.

Conclusions: Target identification and hit to lead medicinal chemistry is now expected to deliver optimized molecules that will then be evaluated in experimental murine models of NASH.
(Funding: PTDC/BIM-MEC/0895/2014; SAICTPAC/0019/2015; SFRH/BD/110672/2015)

Disclosure of Interest: None Declared

P07-10

Adipokine levels and receptor profile as a key modulator of NAFLD susceptibility and severity: A northeast Indian population based study

Sujoy Bose[1], Moumita Bose[*1], Tarun K. Basumatary[2], Purabi D. Bose[2], Anjan K. Saikia[3]*
[1]Biotechnology, Gauhati University, [2]Molecular Biology and Biotechnology, Cotton University, [3]Gastroenterology and Medicinal unit, Central Hospital, NF Rly, Guwahati, India

Corresponding author's email: sujoybose1@gmail.com

Introduction: Role of adipokines in regulation of multiple parameters relating to normal liver physiology has been underlined, with available data on its involvement in NAFLD pathogenesis is far from being conclusive.

Aims: To delineate the role of specific adipokines and their receptors in the pathogenesis of NAFLD in ethnically distinct northeast Indian people.

Material and Methods: Clinically proven non-obese NAFLD cases (n=128) and healthy controls (HC, n=200) with detail clinical profile based on biochemical and fibroscan (LSM-score) data. Panel of serum adipokine levels were measured by multiplex or direct ELISA. Differential mRNA expression profile of specific adipokines receptors was studied by RTPCR using B-actin as normalization control.

Results: Adipokine Leptin and adiponectin levels were found to be deregulated in NAFLD cases compared to controls; while the expression of adipokines Lipocalin-2/NGAL (p=0.867), resistin (0.762), adipsin (p=0.912), PAI-1(total) (p=0.861) was comparable between the groups. The Leptin levels were significantly higher in NAFLD cases (320.1±115.94pg/ml) compared to HC (280.76±67.82 pg/ml) (p=0.013). Serum leptin levels were significantly higher in females than males (p<0.001), but the results of difference in leptin levels were consistent in both sexes. Adiponectin levels were downregulated in NAFLD cases (3.78±1.87µg/ml) compared to HC (5.29±1.65µg/ml) (p=0.087). Serum Leptin levels inversely correlated with adiponectin profile {Pearson correlation= -0.359, p=0.182; Spearman's rho= -0.393,p=0.114}; and positively with LSM-score {Pearson correlation=0.249, p=0.443; Spearman's rho=0.288, p=0.376}, SGOT, SGPT levels in NAFLD cases. Adiponectin levels correlated significantly inversely with LSM-score {Pearson correlation= -0.487, p=0.153; Spearman's rho= -0.632, p=0.050}. Leptin receptor *Ob-Rb* mRNA expression was higher in NAFLD cases (2.78±1.92folds), while the adiponectin receptor *adipoR2* was downregulated in NAFLD (0.319±0.204 folds) compared to controls.

Conclusions: Data is indicative of metabolic deregulation mediated by altered adipokine profile, especially leptin and its receptor *ObRb* upregulation combined with adiponectin downregulation in the susceptibility and severity of NAFLD. Moreover, the lack of adiponectin receptor *adipoR2* results in reduced sensitivity of adiponectin resulting as a combinatorial additive effector of NAFLD pathogenesis. The study underlines the significance of specific adipokines and their receptors as biochemical and prognostic biomarkers respectively.

Disclosure of Interest: None Declared

P08-01

Evaluation of the applicability of non-invasive fibrosis scores in a prospective cohort of non-alcoholic fatty liver disease (NAFLD) patients with liver biopsy

Javier Benavides[*] [1], Nora C. Fernandez[1], Diego Bogetti[2], Luciana Orellano[3], Daniel Pirchi[2], Rafael Maurette[2], Silvina Paz[1], Mariano Bregante[2], Daniela Speisky[4], Maria T. García de Dávila[4], Luis A. Colombato[1]

[1]Gastroenterology Section / Hepatology Unit, [2]Surgery, [3]Hepatology Unit, [4]Pathology, Buenos Aires British Hospital, Buenos Aires, Argentina

Corresponding author's email: benavides.jhb@gmail.com

Introduction: NAFLD progression relates to fibrosis deposition. Liver biopsy (LB), being the gold standard for fibrosis evaluation, is not devoid of sampling errors, costs and morbidity. Non-invasive fibrosis scores are employed to decide LB. However, the performance of such scores is less than optimal

Aims: To evaluate the utility of BARD, Fib4 and NAFLD fibrosis score (NFS) in a cohort of NAFLD patients (pts) with LB

Material and Methods: NAFLD pts with LB were prospectively recruited and followed up in an university hospital in Argentina. Pts with significant fibrosis (SF) were compared with pts without SF (X^2 and t-test). AUROCs, sensitivity (Sen), specificity (Spe), positive and negative predictive values (PPV and NPV) were evaluated for BARD, Fib4 and NFS for the entire cohort and for subpopulations with clinical conditions not included in such scores, i.e. hypertension and metabolic syndrome (MS) for the three scores and diabetes for Fib4
Results: 245 pts included. Age: 52; males 56%; BMI 32; diabetes 25%; MS 65%; hypertension 38%. Simple steatosis 131 pts; NASH 114; SF (F3/F4) 42. Pts with SF were older (61 vs 50 y), had higher % of diabetes (71 vs 15%), hypertension (59 vs 34%) and MS (83 vs 62%) and lower albumin (3,9 vs 4,2 g/dL) and platelets (154 vs 245 x10^9/l) than pts without SF ($p<0.0001$)

ALL PTS.: BARD: AUROC 69% (95% CI 63-75%); Sen 83%; Spe 56%; PPV 28%; NPV 94%. Fib4: AUROC 89% (95% CI 84-94%); Sen 85%; Spe 94%; PPV 72%; NPV 97%. NFS: AUROC 90% (95% CI 85-95%); Sen 88,5%; Spe 93%; PPV 72%; NPV 98%.
HYPERTENSION: BARD: AUROC 69% (95% CI 59-78%); Sen 92%; Spe 46%; PPV 39%; NPV 94%. Fib4: AUROC 97% (95% CI 89-99%); Sen 100%; Spe 94%; PPV 84%; NPV 100%. NFS: AUROC 92% (95% CI 81-97%); Sen 100%; Spe 84%; PPV 73%; NPV 100%
MS: BARD: AUROC 71% (95% CI 63-78%); Sen 91%; Spe 52%; PPV 34%; NPV 96%. Fib4: AUROC 93% (95% CI 87-97%); Sen 95%; Spe 92%; PPV 74%; NPV 99%. NFS: AUROC 94% (95% CI 87-98%); Sen 100%; Spe 88%; PPV 72%; NPV 100%.
DIABETES: Fib4: AUROC 95% (95% CI 82-99%); Sen 95%; Spe 94%; PPV 95%; NPV 94%.
In pts without hypertension, MS or diabetes the performance of the scores was significantly poorer (data not shown), suggesting that these features improves the results of the scores.

Conclusions: In this cohort with low prevalence of SF, NFS and Fib4 yields better results than BARD, with good PPV and very high NPV. Fib4 is a more accurate predictor in diabetics. AUROCs for Fib4 and NFS in hypertension or MS were > 90% with 100% NPV. Diabetes, MS and hypertension help in the management of NAFLD pts.

Disclosure of Interest: None Declared

P08-02YI

A mobile application for the management and follow-up of patients with Non-Alcoholic Fatty Liver Disease

Roberta Forlano[*] [1], Benjamin H. Mullish[1], Nikolaos Katertsidis[2], Nikolaos Giannakeas[3], Alexandros Tzallas[3], Markos Tsipouras[4], Michael Yee[1], Simon Taylor-Robinson[1], Mark Thursz[1], Pinelopi Manousou[1]

[1]Department of Hepatology, Imperial College, London, United Kingdom, [2]Departmenf of Computer Engineering, University of Ioannina, Greece, [3]Departmenf of Computer Engineering, Technological Educational Institute, Epirus, [4]Department of Informatics and Telecommunication Engineering, Greece, Greece, Greece

Corresponding author's email: robertaforl@gmail.com

Introduction: Non-alcoholic fatty liver disease (NAFLD) represents an increasing cause of chronic liver disease and its prevalence has grown with obesity, sedentary lifestyle, unhealthy diet and metabolic syndrome. First line treatment is a combination of dietary modifications and increased physical activity.

Aims: To address the challenge of empowering patients and clinicians to better manage lifestyle, we have designed a novel mobile application entitled F for Fitness.

Material and Methods: We have developed an integrated solution for patients with NAFLD consisting of: A mobile application for coaching and monitoring patients and a cloud-based web application for the management and the storage/analysis of data. The central web application features intelligent issue tracking and a Powerful web analytics providing real-time track of users and their behavior. It is based on Apache Cordova, HTML5, JavaScript and supporting Android, iOS, Windows Phone.

Results: We prototyped and developed an Android app accessible to the patients and a central platform accessible to the clinicians. The app consists of five main screens: Exercise, Food and Water Intake, Alcohol Consumption and Weight/BMI. Exercise is expressed in steps, meters and calories/day. Each meal is recorded choosing between visual options, ranging from raw ingredients to processed food. Similarly, patients enter the quantity and quality of alcohol consumed. In order to give an educational feedback, nutritional facts are displayed after each choice and a pie chart shows the amount of calories consumed vs burned at the end of the day. Finally, each section tracks the history from the beginning of the use of the app. Built-in sensors and third-party devices could fit in easily to manage health and environmental records such as heart rate, blood pressure, glucose levels. Through the central platform, clinicians can access to the records and analyze patients' diet, food intake/exercise balance and alcohol habits. Moreover, each record is pre-filled with clinical data, such as latest Liver Function tests, Liver Stiffness Measurement and Histology report. Push notifications could be send to the patient by the operator, when necessary.

Conclusions: F for Fitness is a novel app prototype in NAFLD. This app acts as an educational tool for the patients and as a real-time follow up of lifestyle for the clinicians, allowing for a personalized management. F for Fitness might be used in clinical practice and in future studies to monitor the effectiveness of behavioural programs.

Figure:

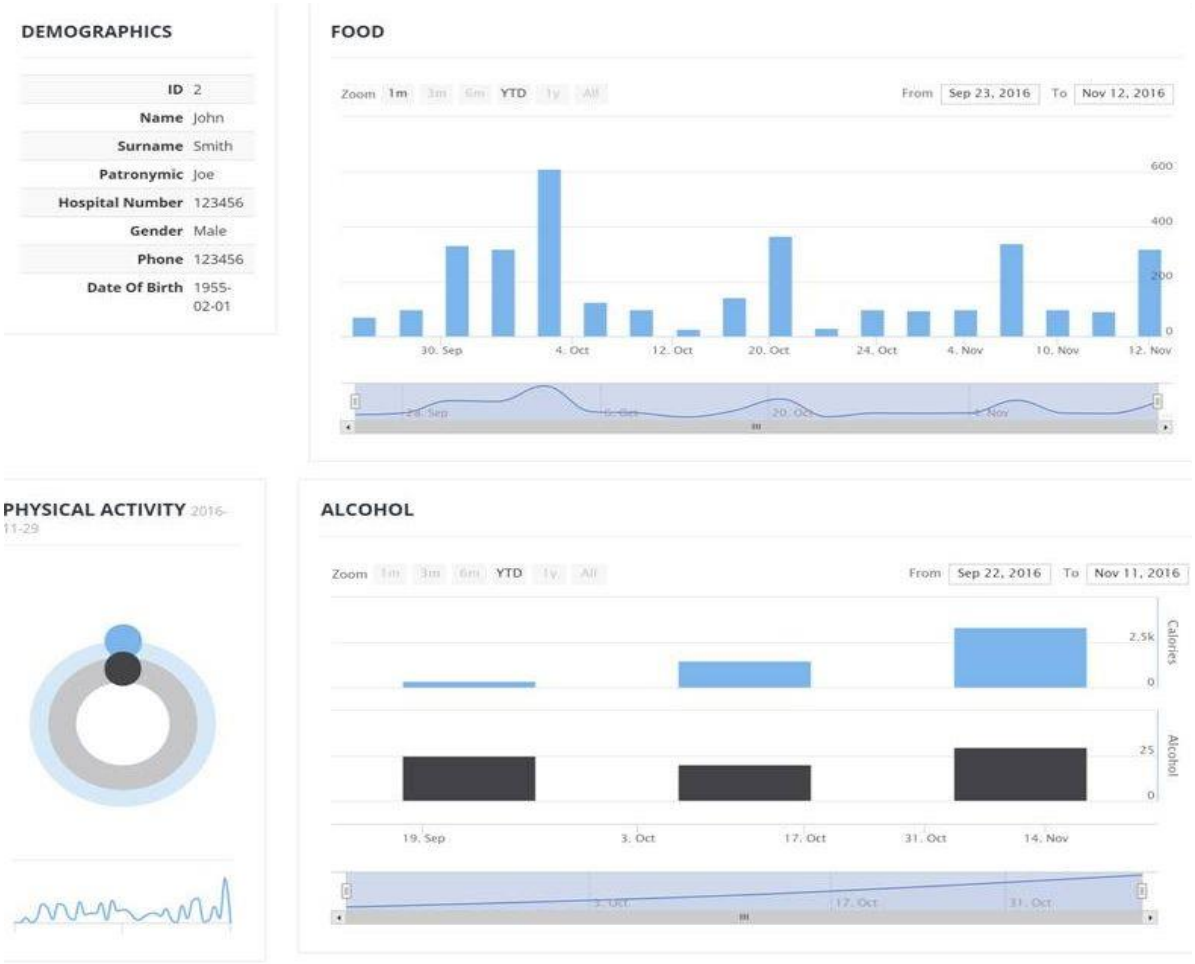


Figure 1. F for fitness: central platform

Figure:

Figure 2. F for fitness: patient's interface

Disclosure of Interest: None Declared

P08-03YI

Comparative characteristics of clinical manifestations and metabolic disorders in men and women with non-alcoholic fatty liver disease

Saidrakhim N. Lukmonov[*] [1], Kurbonboy A. Madatov[1], Muzaffar U. Ismailov[2], Uktam M. Kurbankulov[1], Uktam S. Ismailov[1], Oleg P. Mukhin[3], Oybek O. Usmonov[1], Islom D. Khaydarov[1]

[1]faculty surgery, Tashkent Medical Academy, [2]faculty surgery, Tashkent Pediatric Medical Institute, [3]Gastroenterology, Samarkand State Medical Institute, Tashkent, Uzbekistan

Corresponding author's email: saidrakhim.lukmonov@mail.ru

Aims: This report is based on clinical and statistical parameters, analysis of causal factors contributing to the manifestation of the disease and laboratory indicators, assess in comparative terms in men and women the clinical features of non-alcoholic fatty liver disease.

Material and Methods: A total 90 patients of aged 34-64 years (mean age 50.1±1.4 years) with confirmed diagnosis of NAFLD were examined. It was examined on the basis of the faculty surgery of the Tashkent Medical Academy. There were 43 men (mean age 50.2±1.3 years), women 47 (mean age 50.1±1.5 years).All patients were excluded from professional, oncological, hereditary pathology, chronic alcohol intoxication and blood disease.

Results: MS was verified in all examined patients. At the same time, there were no significant differences ($p>0.5$) in the main criterion–abdominal obesity: waist circumference in men 102.4±2.3, in women-109.8±4.4. However, the body mass index in women was reliably ($p<0.02$) higher, 31.2±0.9 kg/m2 and 28.9±0.9 kg/m2, respectively. Another component of MS–arterial hypertension ($p<0.02$) was detected reliably more often in men, respectively, in 90.7% and 72.3% of cases, but a severe and high risk class was 1.5 times more common in women. It should be noted that type 2 diabetes mellitus was 1.5 times more frequently detected in women. Hypertriglyceridemia was reliably detected ($p<0.005$) in women in 93.6% and 67.4% of cases, respectively, but the degree of its expression was the same for men (3.8±0.4 mmol/l) and women (4.1±0.6 mmol/l). Disorders in the metabolism of porphyrins may be manifested by the appearance of additional combinations of biochemical features: an increase in the content of porphyrins in feces, secondary coproporphyrinuria, chronic latent hepatic porphyria. These biochemical syndromes, in our opinion, characterize the formation of chronic hepatic porphyria.

Conclusions: Thus, the NAFLD in men and women has no fundamental differences in the main clinical and metabolic manifestations. Type 2 diabetes is much more common in women, and arterial hypertension is more reliably detected in men. Hypertriglyceridemia was significantly more frequently detected in women. A different character of the changes is observed with the dismetabolism of porphyrins. Violations are more often reported in men, but the variants of violations are identical.

Disclosure of Interest: None Declared

P08-04

A novel 3D liver microtissue model for studying steatosis in vitro

Fabrice Müller[1], Tobias Strassfeld[1], Simon Messner[*1], Radina Kostadinova[1], Burcak Yesildag[1], Wolfgang Moritz[1], David Fluri[1]

[1]Insphero Ag, Schlieren, Switzerland

Corresponding author's email: simon.messner@insphero.com

Introduction: Hepatic steatosis is characterized by the accumulation of lipid droplets in the liver. According to the "two hit" hypothesis, inflammation can trigger further progress to steatohepatitis, followed by fibrosis and eventually cirrhosis. In vivo, the process needs time (several weeks/months) and requires presence of inflammatory cells and activation of liver non-parenchymal stellate cells into myofibroblasts. Current in-vitro assays, e.g. employing HepG2-cells, are hardly able to mimic the first step towards steatohepatitis, since they lack immune competence and are not suited for long-term exposure. We recently developed a novel 3D liver microtissue model consisting of primary human hepatocytes in co-culture with non-parenchymal cells, exhibiting long-term viability and liver-specific functionality, potentially overcoming many common limitations of current in vitro model systems.

Aims: Development and characterization of a 3D steatosis model as a novel tool to study lipid uptake, storage and depletion.

Material and Methods: 3D Human Liver Microtissues were treated with various concentrations of fatty acids. Several time points were taken and processed for confocal imaging with nile-red fluorescence. Quantification of fluorescence was obtained with Cellprofiler software.

Results: Induced steatosis in liver microtissues was assessed by confocal imaging of nile-red stained lipid droplets. Oleic acid induced time- and concentration dependent lipid accumulation, preferentially causing microvesicular steatosis. The highest lipid accumulation was observed after 7 days Oleic acid treatment. Palmitate induced steatosis, however, exhibited a different pattern of steatosis with larger lipid droplets accumulation, suggesting rather onset of macrovesicular steatosis. The combination of both fatty acids in a physiological relevant 2:1 (Oleic acid:Palmitate) ratio resulted in a mixed phenotype. Quantification of steatosis was achieved by a newly developed algorithm using Cellprofiler software, which showed up to 3-fold induction of lipid droplet accumulation in comparison to the vehicle control.

Conclusions: This results suggest that 3D liver microtissues are a suitable model for studying steatosis in vitro.Further experiments will test the progression of steatosis to steatohepatitis by stimulation of Kupffer cells with inflammatory agents. In addition, anti- steatotic and anti-inflammatory drugs will be assessed for their potency to inhibit disease progression.

Disclosure of Interest: None Declared

P08-05

The combination of Berberis Aristata, Elaeis Guineensis and Coffea Canephora extracts improves insulin resistance in an animal model of NAFLD induced by high fat diet

Vincenzo Lembo[*] [1], Giovanna Mazzone[1], Antonella Rossi[1], Giuseppe D'Argenio[1], Valentina Cossiga[1], Cecilia Nigro[2], Claudia Miele[2], Francesco Beguinot[2], Nicola Caporaso[1], Filomena Morisco[1]

[1]Department of Clinical Medicine and Surgery, Gastroenterology Unit, [2]URT-GDD, National Council of Research & Department of Translational Medical Sciences, University of Naples Federico II, Naples, Italy

Corresponding author's email: v.lembo@hotmail.it

Introduction: Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver disease affecting a large part of the world population. It is well documented that NAFLD is associated to metabolic syndrome. The pathogenesis of NAFLD and metabolic syndrome seems to have insulin resistance as a common pathophysiological mechanism.

Aims: The aim of this study is to evaluate the effect of a mixture of plant extracts consisting of Berberis Aristata, Elaeis Guineensis and Coffea Canephora on the modulation of insulin resistance in an animal model of NAFLD induced by the high fat diet.

Material and Methods: The experiment was conducted for 24 weeks. Six groups of C57BL/6 mice (n=8 each) were randomized into one of the following diets: 1) standard diet (SD, 3% fat); 2) high fat diet (HFD, 60% fat); 3) HFD enriched with plant extract (HFD+E) (140 mg/Kg/die); 4) HFD, and starting from the 13th week SD; 5) HFD, and starting from the 13th week HFD+E; 6) HFD, and starting from the 13th week SD+E. At the end of experiment all mice were fasted for 4 hours, weighted and injected intraperitoneally with a bolus of insulin to evaluate intraperitoneal insulin tolerance test (ipITT). Blood glucose levels were measured at 0, 15, 30, 45, 60, 90 and 120 min after insulin injection from whole tail vein blood using a self-monitoring device.

Results: The HFD mice displayed a significant weight gain compared to SD and HFD+E mice ($p<0.001$ and $p<0.001$, respectively). IpITT revealed insulin resistance in HFD mice ($p<0.001$) while in HFD+E mice there was a significant improvement of insulin sensitivity ($p<0.01$) compared to high fat diet alone.

Conclusions: In conclusion, C57BL/6 mice fed a high fat diet develop obesity and insulin resistance, features of NAFLD and metabolic syndrome. HFD+E improves obesity and significantly reduces insulin resistance. These preliminary data suggest that the extract of Berberis Aristata, Elaeis Guineensis and Coffea Canephora can be used as food support for prevention of NAFLD and metabolic syndrome.

Disclosure of Interest: None Declared

P08-06

The diagnostic role of serum PIVKA-II and AFP in viral- and non-viral-related hepatocellular carcinoma

Natthaya Chuaypen[1], Apichaya Khlaiphuengsin[1], Nutcha Pinjaroen[2], Pisit Tangkijvanich[*1] and Research Unit of Hepatitis and Liver Cancer

[1]Department of Biochemistry, Faculty of Medicine,Chulalongkorn University, [2]Radiology, Faculty of Medicine,Chulalongkorn University, Bangkok, Thailand

Corresponding author's email: pisittkvn@yahoo.com

Introduction: Serum protein induced by vitamin K absence-II (PIVKA-II) is a potential tumor marker for hepatocellular carcinoma (HCC). Limited data are available about its diagnostic performance in various etiological factors of HCC.

Aims: The aim of this study was to compare the diagnostic role of serum PIVKA-II in patients with viral- and non-viral-related HCC.

Material and Methods: Two groups of patients with HCC were studied. Group I included 278 patients with hepatitis B virus (HBV) or hepatitis C virus (HCV) infection and group II included 72 patients with alcoholic steatohepatitis (ASH) or non-alcoholic steatohepatitis (NASH). Serum samples for measuring PIVKA-II and alpha-fetoprotein (AFP) levels were collected at initial presentation prior to any treatment modality for HCC.

Results: Group I were younger and had higher percentage of female than group II. At initial diagnosis, group I had larger tumor size and more advanced BCLC staging than group II. A weak correlation between PIVKA-II and AFP levels was found (r=0.374; *P*<0.001; Spearman's correlation). PIVKA-II and AFP levels were significantly correlated with large tumor sizes and advancing BCLC stage. Serum PIVKA-II levels were significantly higher in group I than in group II (24,426 ± 58,275.6 vs. 6,579.2 ± 17,153.2, *P*<0.001). However, there was no difference between groups regarding AFP levels (20,350.6 ± 62,848.6 vs. 25,743.9 ± 70,727.7, *P*=0.528). Among patients with early BCLC stage (stage 0, A), 27/61 (44.3%) in group I and 14/18 (77.8%) in group II had an elevated AFP level (≥20 ng/mL) (*P*=0.012), while 31/61 (50.8%) in group I and 1/18 (5.6%) in group II had an elevated PIVKA-II level (≥60 mAU/mL) (*P*=0.001).

Conclusions: Serum PIVKA-II could be a better marker for diagnosis of early HCC in patients with chronic viral hepatitis. In contrast, serum AFP had a better performance for early detecting HCC in patients with underlying ASH/NASH.

Disclosure of Interest: None Declared

P08-07YI

Is non-obese fatty liver disease characterized with the same to typical NAFLD features or can be recognized as distinctive condition?

Nazarii Kobyliak[1], Natalia Bosak[*1], Galyna Mykhalchyshyn[1], Dmytro Kyriienko[2], Oleg Dynnyk[3]

[1]Bogomolets National Medical University, [2]Kyiv City Clinical Endocrinology Center, [3]Bogomolets Institute of physiology of the Ukrainian National Academy of Sciences, Kyiv, Ukraine

Corresponding author's email: natusiabosak@gmail.com

Introduction: Clearly, not all obese subjects develop non-alcoholic fatty liver disease (NAFLD) and NAFLD can also be found in non-obese patients. Globally, the reported prevalence of non-obese NAFLD varies widely, ranging from 3% to 30%. Today remains unclear how patients without obesity develop NAFLD, therefore it is important to understand the pathological conditions of non-obese NAFLD.

Aims: In this study, we investigated the clinical and metabolic parameters in type 2 diabetes (T2D) patient with non-obese and obese NAFLD detected on ultrasonography (US).

Material and Methods: In this cross-sectional study, 245 T2D patients with age of 40–80 years from the Kyiv City Clinical Endocrinology Center were selected. Inclusion criteria were: age over 18 years, presence of T2D in association with fatty liver disease. The diagnosis of fatty liver was based on the results of abdominal ultrasonography, which was done by trained technicians with Ultima PA (Radmir Co., Ukraine). According to body mass index (BMI) patients were assigned else to NAFLD group (n=157, BMI≥30.0kg/m^2) or to non-obese NAFLD (n=88, BMI<30.0kg/m^2) group. We measured liver stiffness (LS) by using Shear Wave Elastography (SWE) technique and calculated fatty liver index (FLI) designed by Bedogni et al. Changes in transaminases activity, serum lipids and cytokines (TNF-α, IL-1β, IL-6, IL-8, INF-γ) levels were evaluated.

Results: Non-obese NAFLD patient had higher LS (7.52±0.2 vs 6.87±0.09, p=0.001) values measured by SWE, which were accompanied with increased transaminase activity. LS significantly correlated with ALT (r=0.482, p=0.007) and AST (r=0.404, p=0.027) respectively. In contrast to non-obese group, patient in NAFLD group had significantly higher FLI (86.59±1.09 vs 68.06±1.98, p<0.001), which were also associated with LS value (r=0.247, p=0.022). Markers of chronic systemic inflammatory state were also significantly higher in NAFLD obese as compared to non-obese patient: IL-1β – 44.64±2.0 vs 31.02±1.78 (p<0.001); TNF-α – 54.11±2.20 vs 42.28±1.81 (p<0.001); IL-8 – 29.18±1.27 vs 22.05±0.99 (p<0.001) and INF-γ – 195.60±9.47 vs 132.47±7.54 (p=0.016) respectively. Changes of IL-6 between groups were insignificant.

Conclusions: We found that non-obese NAFLD associated with higher liver stiffness values and transaminases activities. On the other hand, patient with obese NAFLD are characterized with more pronounced liver fat content and elevation of markers of chronic systemic inflammatory state.

Disclosure of Interest: None Declared

P08-08YI

Reduction of weight and levels of noninvasive biomarker of liver apoptosis in patients with nonalcoholic fatty liver disease by lifestyle modification program during 6 months

Nataliia Dynnyk[1], Olena Baka[*2], Elina Manzhalii[3]

[1]Gastroenterology, Bogomolets National Medical University, [2]Gastroenterology, the State Scientific Institution "The Center of Innovative Medical Technologies of the National Academy of Science of Ukraine", [3]Propedeutics of Internal Medicine II, Bogomolets National Medical University, Kyiv, Ukraine

Corresponding author's email: dynka@ukr.net

Introduction: Lifestyle modification and weight loss above 7-10 % is the most effective treatment of NAFLD. But the big challenge is getting the patient´s compliance with dietary recommendations and physical activity.

Aims: The aim of this study was to estimate the role of lifestyle modification program during 6 months in weight loss in patients with NAFLD. Also we discovered how this program can improve hepatic apoptosis as a main predictor of Nonalcoholic fatty liver disease progression, which can be measured by biomarker cytokeratin 18.

Material and Methods: 58 patients with NAFLD were randomized in a two groups. All of them had diagnosed NAFLD. All patient´s were informed about importance of weight loss for the improving NAFLD. We gave diet recommendations which consists of reducing portions of food, avoiding drinking soda water, reducing carbohydrates intake and increasing fiber intake. Increasing physical activity consists of walking 12000 steps per day. For better diet adherence every day first group were writing food diaries and for physical activity compliance they used pedometers. Each person from first group had 6 personalised sessions (once per month) with dietitian and 2 individual consultations with psychologist during 6 months. On each sessions with dietitian patients were measured of weight, waist circumference and fat percentage measured by bioelectric impedancemetry. To those from control group were just given general diet recommendations and physical activity recommendations without controlled sessions with dietitian and psychologist during 6 months.

Results: We observed significant decreasing in weight loss ($p<0,01$), cytokeratin 18 fragments M30 ($p<0,01$), Fatty liver index, Visceral adiposity index and Fat contain measured by bioelectric impedancemetry in patients from first group that were controlled in lifestyle modification program during 6 month.

Conclusions: Lifestyle modification program with 6 dietitian and 2 psychologist sessions during 6 month leads to weight loss and improves levels of non-invasive marker of liver inflammation and apoptosis Cytokeratin 18 in patients with NAFLD.

Disclosure of Interest: None Declared

P08-09

Frequency of non alcoholic fatty liver diasease in patients with diabetes mellitus

Nauman A. Jadoon*[1, 2], Athesham Zafar[3], Najeeb Shah[4]

[1]Medicine, Ittefaq Hospital, Lahore, Pakistan, [2]Medicine, [3]Gastroenterology, [4]Hull Royal Infirmary, Hull, United Kingdom

Corresponding author's email: dr.naumanjadoon@gmail.com

Introduction: Nonalcoholic fatty liver disease (NAFLD) is commonly associated with type 2 diabetes mellitus (DM) though its prevalence is not well studied. This was a prospective study of prevalence and risk factors of NAFLD in patients with type 2 diabetes.

Aims: The aim of this study was to determine the frequency of NAFLD in patients with T2DM.

Material and Methods: A total of 200 patients with T2DM fulfilling the criteria were enrolled after taking informed consent in Fall 2016/Spring 2017. Thorough medical history and relevant physical examination was taken. Demographic data such as age, gender were noted. Ultrasound was done and then patients were classified as having NAFLD if they had evidence of steatosis with no causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication, or hereditary disorders.

Results: The mean age of patients was 52.40 years with standard deviation of 2.68 years with most of the patients in the age range 45-60 years. Majority of the patients in the study were female (62.0%). Mean duration of DM was 6.2 years with standard deviation of 2.58. Mean fasting plasma glucose was 145.5±5.36 mg/dl. Frequency of NAFLD, as per operational definition was 55%. Stratification of data with respect to age, gender, duration of diabetes and treatment of diabetes show P value was >0.05 in all cases showing statistically insignificant difference between various subgroups.

Conclusions: Prevalence of NAFLD in our cohort of type 2 DM patients is high. It can occur in diabetic patients without any symptoms, signs or routine laboratory test abnormalities and thus needs to be screened.

Disclosure of Interest: None Declared

P08-10YI

Attenuation coefficient measurement (ACM) as novel real time ultrasound alternative to cap (FibroScan)

Nazarii Kobyliak[*] [1], Nataliia Marunchyn[1], Olexander Fedusenko[2], Vitaliy Gurianov[1], Oleg Dynnyk[3]

[1]Bogomolets National Medical University, [2]Shupyk National Medical Academy of Postgraduate Education, [3]Bogomolets Institute of physiology of the Ukrainian National Academy of Sciences, Kyiv, Ukraine

Corresponding author's email: nazariikobyliak@gmail.com

Introduction: The presence of fat droplets in the hepatocytes (micro- or macrovesicular hepatic steatosis) under condition of chronic diffuse liver disease (CDLD) increases the attenuation of ultrasound (US). A group of Ukrainian scientists proposed an original algorithm for real-time US attenuation measurement (attenuation coefficient measurement – ACM – patent UA №2014 111234)

Material and Methods: From total of 3274 patients who underwent to comprehensive abdominal US (2015-2016) in our clinic, 949 have been diagnosed with fatty liver according to Hamaguchi criteria. All these patient we provide ACM (dB/cm) measurement on SoneusP7 device (Ultrasign, Ukraine), with a 1–6 MHz convex transducer in the right and left lobes. For diagnostic accuracy assessment (used CT as standard) and comparison with CAP measured by Fibroscan (Echosens, France) we included 142 patients for subanalysis. Evaluation of diagnostic accuracy of ACM performed using ROC-analysis.

Results: Depending on the stage of steatosis according to B-mode median, 25 and 75 quartiles for ACM were as follows: control group 1.57 (1,32-1,85); S1 - 1,86 (1,78 - 2,11); S2 - 2,26 (2,20-2,49) and respectively for S3 - 2,7 (2,40-2,82) dB/cm.
ACM value increase parallel the hepatic steatosis progression ($p<0.001$), which was also accompanied with presence of very strong correlation between these parameters ($r=0,814$, $p<0.001$).
In patient with NAFLD the association between maximum value of ACM and duration of T2DM and triglycerides (model 1, multiple correlation coefficient=0.55; $R^2=0.26$; $p=0,004$) and ALT (model 2, multiple correlation coefficient=0.55; $R^2=0.25$; $p=0,005$) were observed. After adjustment by the duration of T2DM the level of triglycerides ($r=0.44$, $p=0.012$) and activity of ALT ($r=0.44$, $p=0.012$) significantly correlated with ACM.
The AUROC of ACM for steatosis diagnosis was 0,925 (95% CI 0.877-0.973). The optimal cutoff point was >2.27 dB/cm, with sensitivity, specificity, PPV and NPV respectively 91.5, 77.3, 84.6 and 83.8 %.
ACM value also significantly correlated with CAP ($r=0,630$, $p<0.001$).

Conclusions: The ACM as novel real time ultrasound approach can be used for noninvasive hepatic steatosis diagnosis, allows clinicians to monitor disease progression and response to treatment.

Disclosure of Interest: None Declared

P09-01

Nonalcoholic fatty liver disease: effects of human resistin on immunological cells

Cecilia C. García[1], Nadia S. Alegre[1], Placida Baz[1], Javier Benavides[* 2], Luis A. Colombato[2], Daniel Poncino[3], Daniel García[3], Alejandra C. Cherñavsky[1]

[1]Instituto de Inmunología, Genética y Metabolismo, Conicet-UBA, Hospital de Clínicas José de San Martín, [2]Gastroenterology Unit / Hepatology Section, Buenos Aires British Hospital, [3]Gastroenterology Unit / Hepatology Section, Sanatorio Méndez ObSBA, Buenos Aires, Argentina

Corresponding author's email: benavides.jhb@gmail.com

Introduction: Human resistin (hRES) is a cytokine which plasma concentration and hepatic expression have been found elevated in nonalcoholic fatty liver disease (NAFLD) patients. Although hRES is produced by immunological cells, its effects on them are poorly understood. It has been proposed that hRES receptor is adenylyl cyclase-associated protein 1 (CAP1), which expression was only reported in THP-1 monocyte cell line.

Aims: To evaluate hRES-mediated modulation of T cell early (CD69) and late (CD25) activation markers, hRES ability to modulate reactive oxygen species (ROS) production and CAP1 expression in peripheral blood mononuclear cells (PBMC) from NAFLD patients and controls (Co).

Material and Methods: PBMC were obtained from NAFLD patients (n= 23) and Co (n= 35) and evaluated for CAP1 expression. Isolated T cells were activated with coated anti-CD3 (3 ug/ml) +/- hRES (10 ng/ml) for 24 or 72 h and studied for CD69 or CD25 expression. In order to evaluate ROS levels, PBMC were incubated with or without hRES (20 ng/ml) for 24 h and stained with 2´7´-dichlorofluorescin diacetate (DCFH-DA). As a preliminary approach, monocyte oxidative burst was stimulated with phorbol 12-myristate 13-acetate (PMA, 100 ng/ml) +/- hRES and ROS production was evaluated using DCFH-DA. Cells were stained with anti-CD3, -CD4, -CD8 or -CD14 antibodies for T cells or monocytes identification. Protein expression and ROS production were evaluated by flow cytometry. Mann-Whitney and Wilcoxon paired test were used.

Results: Compared with Co, NAFLD patients showed increased CAP1 expression in monocytes ($p<0.05$), $CD4^+$ ($p<0.05$) and $CD4^-$ ($p<0.01$) T cells. hRES decreased CD69 and CD25 expression in activated T cells from Co but not from NAFLD patients. As a result, CD69 and CD25 expression was higher in $CD4^+$ ($p<0.05$; $p<0.01$) and $CD8^+$ ($p<0.01$; $p<0.05$) activated T cells from NAFLD patients. hRES decreased basal ROS levels in monocytes ($p<0.05$), $CD4^+$ ($p<0.001$) and $CD8^+$ ($p<0.001$) T cells only from Co. NAFLD patients showed higher ROS levels than Co in $CD4^+$ ($p<0.05$) and $CD8^+$ ($p<0.05$) T cells.

Conclusions: The presence of hRES prevented ROS production in PMA-stimulated monocytes from Co. hRES can modulate activation markers expression and ROS levels in T cells from Co but not from NAFLD patients. Thus, despite of the higher CAP1 expression in immunological cells from NAFLD patients, an alteration in hRES signaling pathway might contribute to NAFLD progression through ROS production and/or T cell-mediated injury.

Disclosure of Interest: None Declared

P09-02YI

Assessment of non-invasive markers of fibrosis against collagen quantitation and NASH-CRN scoring in liver biopsies of NAFLD patients

Roberta Forlano*[1], Benjamin H. Mullish[1], Nikolaos Giannakeas[2], Alexandros Tzallas[2], Markos Tsipouras[3], Michael Yee[1], Simon Taylor-Robinson[1], Jo Lloyd[4], Robert Goldin[4], Shahid Khan[1], Mark Thursz[1], Pinelopi Manousou[1]

[1]Department of Hepatology, Imperial College, London, United Kingdom, [2]Departmenf of Computer Engineering, Technological Educational Institute, Epirus, [3]Department of Informatics and Telecommunication Engineering, Greece, Greece, Greece, [4]Department for Cellular Pathology, Imperial College, London, United Kingdom

Corresponding author's email: robertaforl@gmail.com

Introduction: Non-alcoholic fatty liver disease (NAFLD) is an increasing cause of chronic liver disease worldwide, with an estimated global prevalence of ~25%. Whilst liver biopsy is regarded as the reference standard for staging, it has significant limitations. Given the huge burden of NAFLD, there is much interest into the role of non-invasive assessment of fibrosis.

Aims: We compare non-invasive markers of fibrosis against semi-quantitative histology and collagen quantitation.

Material and Methods: We retrospectively assessed all consecutive patients with biopsy-proven NAFLD followed-up at the Liver Unit of St. Mary's Hospital, from January 2010 to December 2016. The AST to platelet ratio Index (APRI), BARD Score, FIB-4 and NAFLD Fibrosis Score (NFS) were calculated at the time of liver biopsyand Liver Stiffness Measurements (LSM) were obtained within 3 months from the biopsy. Liver biopsies were both scoredusing semi-quantitative scoring (NASH CRN scoring system) and automated image analysis based on machine learning for the quantitation offibrosis,expressed as Collagen Proportional Area (CPA). AUROCs were calculated to diagnose fibrosis stage and CPA.

Results: 238 patients with median age 50 ±13 years and median BMI 30.7±5.3 kg/m^2 were included. 185 NASH and 118 advanced fibrosis (F3-F4). When semi-quantitative assessment was used as reference, LSM and FIB-4 showed the best AUROCs for diagnosing ≥F3 (0.79,95%IC=0.69-0.91 and 0.75 95%IC=0.62-0.9), followed by BARD score (0.7,95%IC=0.55-0.81), NFS (0.65,95%IC=0.54-0.73) and APRI (0.6, 95%IC=0.5-0.69) (Figure 1). Using quantitative assessment as reference, LSM and NFS performed better (AUROCs 0.77,95%IC=0.63-0.89 and 0.71,95%IC=0.62-0.79) to diagnose CPA>5%, followed by BARD score (0.7,95%IC=0.61-0.78), FIB-4 (0.65,95%IC=0.49-0.69) and APRI (0.51,95%IC=0.39-0.61) (Figure1). Similarly, LSM and NFS AUROCS to diagnose CPA≥12% were 0.77 (95%IC=0.69-0.75) and 0.71 (95%IC=0.65-0.82), followed by FIB-4 and BARD (0.62,95%IC=0.51-0.62 and 0.61,95%IC=0.5-0.61) (Figure 2). PPV have been overall below 60%, while LSM and NFS showed excellent NPV: for CPA≥5% (NPV 81% and 88%) and for CPA≥12% (NPV 87% and 89%).

Conclusions: LSM remains the best surrogate marker for predicting advanced fibrosis. Quantitation of collagen in liver biopsies suggests that NFS performs better than FIB-4, with the latest performing better when NASH-CRN scoring is used. An algorithm involving both NFS and FIB-4 score should be used to stratify patients at risk of advanced fibrosis.

Figure:

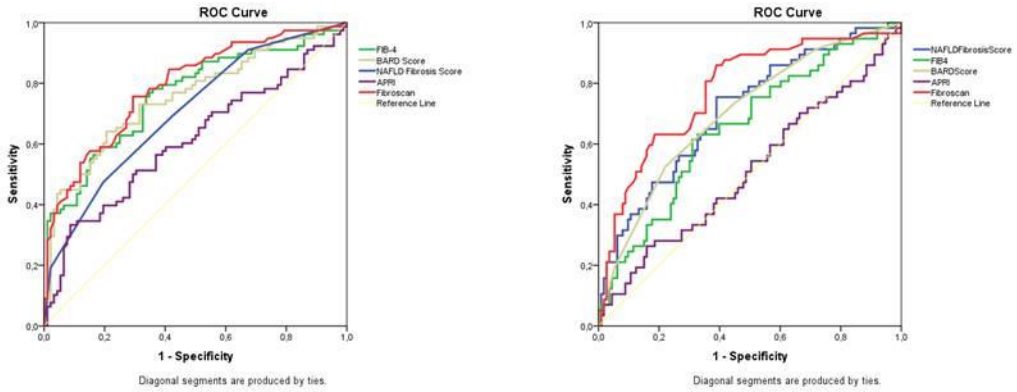


Figure1. AUROCs for non-invasive markers to diagnose fibrosis ≥F3 (on the left) and CPA≥5% (on the right)

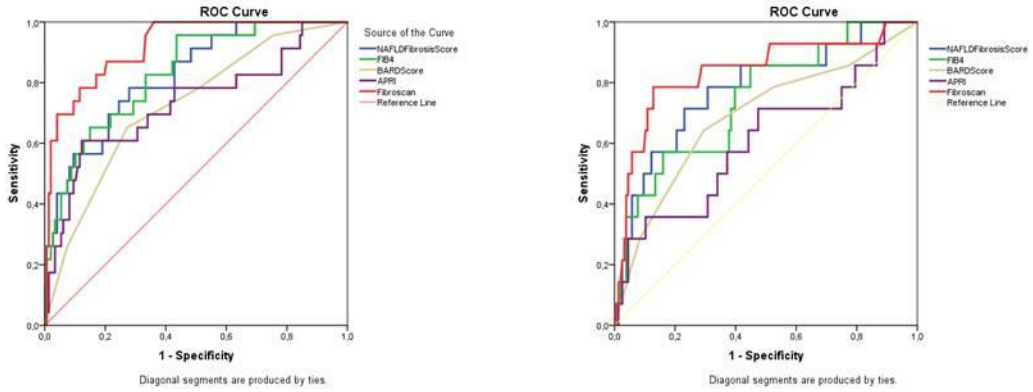


Figure2. AUROCs for non-invasive markers to diagnose fibrosis ≥F4 (on the left) and CPA≥12% (on the right)

Disclosure of Interest: None Declared

P09-03

Comparative assessment of different diagnostic scores for prediction of non-alcoholic liver disease

Nevena E. Kojić [*1,2], Mirjana Đerić[1,2], Mira Govorčin[2,3], Dragana Balać[4], Milena Kresoja[5]

[1]Centre for Laboratory Medicine, Clinical Centre of Vojvodina, [2]Medical Faculty , University of Novi Sad, [3]Centre for Radiology, Clinical Centre of Vojvodina, [4]Centre for Hygiene and Human Ecology, Institute of Public Health of Vojvodina, [5]Department of Mathematics and Informatics, Faculty of Sciences, University of Novi Sad, Novi Sad, Republic of Serbia

Corresponding author's email: nevenaer@gmail.com

Introduction: Several steatosis risk score were developed in order to optimize identification of persons with NAFLD (fatty liver index- FLI, hepatic steatosis index- HSI).

Aims: Our aim was to determine which one is most applicable to our study group.

Material and Methods: 77 non-smokers with abdominal obesity without cardiovascular diseases, renal diseases, infective, malignant and autoimmune diseases. Anthropometric parameters, markers of glucose and lipid metabolism, serum levels of inflammatory markers, levels of liver enzymes, as well as ferritin and uric acid were assessed in all subjects. Fatty liver was assessed as presence or absence and grading of hepatic steatosis obtained by ultrasound scan using National Health and Nutrition Examination Survey (NHANES) III.

Results: Anthropometric parameters as well as liver enzymes, uric acid and hsCRP were significantly higher in patients with NAFLD (BMI 28.05±4.79 vs 34.38±9.73 kg/m2, p=0.001; WC 96.15±14.27 vs 108.05 ± 11.47 cm, p=0.001; SBP 122.42±10.62 vs 128.98 ±8.67 mmHg, p=0.01; DBP 78.33±7.57 vs ± 5.94 mmHg, p= 0.001; hsCRP 1.98± 2.34 vs 4.34±5.56 mg/l, p=0.004; uric acid 296.76±74.06 vs 358.02±83.29 µmol/l, p=0.001; AST 21.70±5.21 vs 23.93±6.91 U/L, p= 0.014; ALT 23.00 ± 11.75 vs 30.50 ±13.70 U/L, p= 0.007). Factor derived from factor analysis that had incorporated waist circumference, hip circumference, body mass index, systolic and diastolic blood pressure, fibrinogen, hsCRP, glucose and uric acid had best discriminatory power followed with FLI and HSI.

Conclusions: Further trials are needed to adjust existing steatosis risk scores and incorporate other markers of steatosis such as uric acid.

Disclosure of Interest: None Declared

P09-04YI

Unbiased selection of NAFLD animal model using hepatic whole-genome transcriptomic meta-analysis

Nicolas Goossens[*] [1], Laurent Spahr[1], Petros Tsantoulis[2], Jean-Louis Frossard[1], Francesco Negro[1, 3]

[1]Division of Gastroenterology & Hepatology, Geneva University Hospital, Geneva, [2]Division of Oncology and Swiss Bioinformatics Institute, Geneva University Hospital, Geneve, [3]Division of Pathology, Geneva University Hospital, Geneva, Switzerland

Corresponding author's email: nicolas.goossens@gmail.com

Introduction: No mouse models of non-alcoholic fatty liver disease (NAFLD) fully recapitulates the entire phenotype of this complex disease. An unbiased approach to identify the most suitable animal model of NAFLD, in particular linked to a specific molecular pathways/deregulations of interest would be a valuable tool for translational research and identification/validation of novel targets.

Aims: We performed an unbiased whole-genome transcriptomic meta-analysis to identify animal models most closely related to human NASH in particular advanced fibrosis in NAFLD.

Material and Methods: We assessed human hepatic transcriptome profiles and 7 mouse models of NAFLD (early, late and overall high-fat [HF], choline- and folate-deficient [CFD], methionine and choline deficient [MCD], HF, high-cholesterol, high-cholate [CL], HF high-sucrose [HSHF] diets, and 2 genetic models: PTEN-liver-specific knock-out (PKO) and leptin-deficient mice (obob). We compared the animal and human hepatic transcriptome by gene expression meta-analysis, principal component analysis (PCA) and hierarchical clustering (HC). Molecular pathways were analyzed by gene set enrichment analysis (GSEA).

Results: We identified 10 human studies comparing human NASH versus healthy controls, including 128 NASH subjects and 134 healthy controls. 123 genes were differentially expressed in either of the human meta-analyses (FDR < 0.05 and beta > 1.0) of which 71 genes (58%) were differentially expressed in at least one animal model. At the gene expression level, the CL, CFD, PKO and obob mice had the greatest correlation to NAFLD fibrosis (ρ>0.3) and the obob, HSHF, CL, PKO and HFD mice had the closest correlation to human NASH. Using HC and PCA at gene and pathway level, no single animal clearly reproduced the transcriptional deregulations of human NAFLD. However, our approach indicated that some diets reproduced key pathways induced in human NASH or NAFLD fibrosis for example fatty acid metabolism was suppressed in NAFLD fibrosis, similar in CFD, CL or MCD diets, but induced in NASH subjects, similar to HSHF and HF diets.

Conclusions: Although no animal model perfectly recapitulates the complex molecular deregulations seen in human NAFLD, our approach allows an unbiased choice of the most suitable NAFLD mouse model depending on the molecular deregulations of interest.

Disclosure of Interest: None Declared

P09-05

Efficacy of the obesity treatment with a probiotic, special diet and metabolic conditioning complex (MetCon)

Elina Manzhalii[1], Olena Baka[*2], Taras Nykula[1], Valentyna Moyseyenko[1], Vitalii Kondratiuk[1]

[1]Propedeutics of Internal Medicine II, Bogomolets National Medical University, [2]gastroenterology, The Center of Innovative Medical Technologies of the National Academy of Sciences of Ukraine, Kyiv, Ukraine

Corresponding author's email: olenabaka@yahoo.com

Introduction: Obesity treatment is supposed to be complex and it is necessary to take into account a lot of factors which cause the obesity.

Aims: Our aim was to evaluate the efficacy of the combined therapy with the probiotic the E.coli strain Nissle 1917 (EcN), a special low carbohydrates diet and MetCon as obesity treatment.

Material and Methods: In an open label trial, 57 patients with obesity were divided into two groups: control group (CG) of 30 patients being on a diet and doing exercises MetCon and an experimental group (EG) of 27 patients receiving in addition EcN twice daily for 3 months. The presence of the NASH was observed in 96%. There were 35 women and 22 men aged 23-53 years (mean age - 41 ± 3,1 years). The average BMI in 69% of patients was 31,2 ± 2,1 (first grade of obesity), 31% of patients -with the second grade of obesity had BMI 36,4 ± 3,1. The content of leptin in serum of patients before treatment was 39,7±2,5 ng/ml. Improvement of NASH was evaluated by determination of transaminases, GGT, cholesterol levels and changes of the liver stiffness revealed by ultrasound share wave elastography. The stool microbiota was quantified by standard techniques.

Results: After treatment the BMI of the EG patients with first degree of obesity was 27,1 ± 2,7, the BMI in EG patients EG with the second degree of obesity was 31,8 ± 2,9. Average rate of weight loss was 8,5 ± 1,16 kg compared to CG 4,5 ± 2,2 kg. All patients involved in the trial had a fatty liver diagnosed by ultrasound examination with elevation of GGT and ALT as minimal criteria of NASH and with no differences between the randomly selected patients in the CG and EG. The short-term 3-months NASH treatment of patients with EcN in the EG was accompanied by a significant decrease of of transaminase levels by > 20 %. The stiffness of the liver was decreased in the EG by a mean of 0.7 kPa compared to no change in the CG. Moreover, a decrease in cholesterol was noted in the EG as well as a reduction in body weight. However, the level of leptin decreased in both groups. The stool microbiota of patients in the EG revealed a significant enrichment for all examined bacterial species with the exception of pathogenic enterobacteria compared to CG.

Conclusions: Short-term treatment with a probiotic EcN, special low carbohydrates diet and MetCon could reduce inflammation and weigh in obese patients with NAFLD.

Disclosure of Interest: None Declared

P09-06YI

Parameters of liver and pancreas stiffness in children with nonalcoholic liver disease

Olha Lukianenko[*] [1], Natalya Zavgorodnya[2], Yuriy Stepanov[3], Nina Hravyrovska[4], Yahmur Victoriya[5]

[1]Pediatric gastroenterlology department, "Institute of gastroenterology of the National academy of medical sciences of Ukraine", [2]Pediatric gastroenterlology department, Institute of gastroenterology of the National academy of medical sciences of Ukraine, [3]Institute of Gastroenterology of the National Academy of Sciences, [4]Institute of Gastroenterology of the National Academy of Sciences Ukraine, Dnipro, Ukraine, [5]Pediatric gastroenterology department, Institute of Gastroenterology of the National Academy of Sciences Ukraine, Dnipro, Ukraine

Corresponding author's email: sirenkolga@gmail.com

Introduction: Nonalchoholic liver disease is associated with progressive fibrosis and can be accompanied with nonalcoholic fatty pancreas disease.

Aims: The aim of the study is to establish the features of liver and pancreatic stiffness in children with nonalchoholic liver disease using transient and sharewave elastography.

Material and Methods: Materials and methods. We observed 85 children aged 7 to 17 years old, the average age was (11,830 ± 2,81) years. In order to determine pancreatic fibrosis and steatosis, shear wave elastography and steatometry (quantitative estimation of the ultrasound attenuation with determination of average ultrasound attenuation coefficient (aUAC)) were performed using UltimaPAExpert apparatus ("Radmir", Ukraine). Liver fibrosis and steatosis were diagnosed with the usage of Fibroscan 502 Touch (France) with CAP (controlled attenuation parameter) function. According to presence of the liver steatosis, children were divided into the following groups: 1 group - 30 children with liver steatosis and obesity/overweight; group 2 - 55 children without liver steatosis. All patients and their parents had given their agreement to participation in the study.

Results: Level of the CAP in the 1 group was (261,5±36,67) dB/m and (194,18±37,47) dB/m in the 2 group($p<0,0001$). Average ultrasound attenuation coefficient of the pancreas acquired maximum values in children with pancreatic steatosis, level of UAC was also significantly higher in children of the 1 group (in the 1 group – (2,51±0,32) dB/sm; on the 2 group – (2,28±0,41) dB/sm) ($p=0,039$). We found that level of the liver stiffness measurement was significantly higher in the children with liver steatosis (in the 1 group – (4,29±0,85) kPa, in the 2 group - (3,74±0,79) kPa, ($p=0,004$). Pancreatic stiffness was higher in the patient of the 1 group - (3,75±0,67) kPa and (3,50±0,80) kPa in the 2 group ($p=0,23$). We observed presence of positive correlation between level of aUAC and CAP – $r=0,34$, ($p<0,05$) and negative correlation between pancreatic stiffness and aUAC ($r=--0,28$, $p<0,05$).

Conclusions: Conclusion. The study showed that nonalcoholic fatty liver disease is accompanied by increasing of liver stiffness, while level of pancreatic stiffness didn't show significant differences between groups. Pancreatic stiffness showed negative correlation with average ultrasound attenuation coefficient of the pancreas that can be explained by relative decline of pancreatic stiffness in the case of pancreatic steatosis.

Disclosure of Interest: None Declared

P09-07YI

CCR2+ Infiltrating monocytes promote experimental steatohepatitis - Therapeutic implications of inhibiting CCR2

Oliver Krenkel[*1], Tobias Puengel[1], Jana C. Mossanen[1], Thomas Longerich[2], Eric Lefebvre[3], Christian Trautwein[1], Frank Tacke[1]

[1]Department of Medicine III, [2]Institute of Pathology, RWTH-University Hospital, Aachen, Germany, [3]Allergan, South San Francisco, United States

Corresponding author's email: okrenkel@ukaachen.de

Introduction: Macrophages are key inflammatory cells promoting either progression or regression of non-alcoholic steatohepatitis (NASH). Recent studies suggest that both monocyte-dependent macrophages as well as resident phagocytes, Kupffer cells, promote NASH development by releasing inflammatory mediators. In mice and men, monocytes' egress out of the bone-marrow and their subsequent infiltration into the liver is mediated via the CCL2-CCR2 axis. Cenicriviroc (CVC) is an oral chemokine receptor CCR2/CCR5 antagonist currently evaluated in a phase-2b clinical trial in patients with NASH and fibrosis.

Aims: We tested the efficacy of blocking monocyte recruitment into injured livers by using CVC in mouse models of chronic liver injury progression and regression.

Material and Methods: We studied the effects of CVC on monocyte migration during NASH progression in C57BL/6J wildtype (WT) mice by feeding methionine-choline-deficient (MCD) diet for 8 weeks and high-cholesterol Western diet (WD) for 16 weeks. Treatment with the CCR2/CCR5 antagonist CVC was started after 4 weeks MCD or 8 weeks WD feeding, respectively. RNA sequencing analysis was performed for highly-pure FACS-isolated hepatic macrophage populations from NASH and control mice.

Results: Infiltrating CCR2$^+$ monocytes expanded the population of monocyte-derived macrophages during NASH progression. Therapeutic inhibition of CCR2 and CCR5 by CVC led to a significantly reduced influx of bone-marrow derived CCR2$^+$ monocytes into the liver. CVC treatment significantly ameliorated steatohepatitis, as assessed by histological NAFLD activity score, oral glucose tolerance test and reduced hepatic fibrosis. Therapeutic inhibition of infiltrating monocytes did not delay resolution after injury cessation. Whole genome RNA sequencing analysis revealed that monocyte-derived macrophages, but not Kupffer cells, specifically upregulate multiple growth factors and cytokines associated with fibrosis progression, while Kupffer cells activated pathways related to inflammation initiation and lipid metabolism.

Conclusions: The chemokine receptor CCR2/CCR5 inhibitor CVC potently blocks the infiltration of pro-inflammatory monocytes in experimental models of non-alcoholic steatohepatitis. RNA sequencing analysis revealed that monocyte-derived macrophages express characteristic genes of inflammation and fibrosis progression. Therapeutic inhibition of monocyte influx efficiently ameliorated hepatic inflammation and fibrosis, corroborating the therapeutic potential of CVC in patients with NASH.

Disclosure of Interest: O. Krenkel: : None Declared, T. Puengel: : None Declared, J. Mossanen: : None Declared, T. Longerich: : None Declared, E. Lefebvre: Employee: Allergan, C. Trautwein: : None Declared, F. Tacke: : None Declared

P09-08YI

Hepatic stiffness evaluation with shear wave elastography (SWE) in severe obese patients

Paola Bizzotto[*] [1], Giancarlo Bombonato[1], Silvia Bettini[1], Roberto Fabris[1], Paolo Angeli[1], Roberto Vettor[1], Carlo Merkel[1]

[1]Dipartimento di Medicina, Università di Padova, Padova, Italy

Corresponding author's email: paolabizz@gmail.com

Introduction: Liver steatosis can be diagnosed either using a liver biopsy or by imaging techniques such as ultrasound. The detection of hepatic fibrosis is also useful in the evaluation of the progression of steatosis to cirrhosis. The gold standard technique remains liver biopsy, but it presents several limitations. Transient elastography with Fibroscan is a non-invasive method providing an accurate measure of hepatic fibrosis but is generally not applicable in obese patients. One of the recently developed method in the assessment of liver stiffness is shear wave elastography (SWE) that is claimed to be suitable even in the presence of obesity.

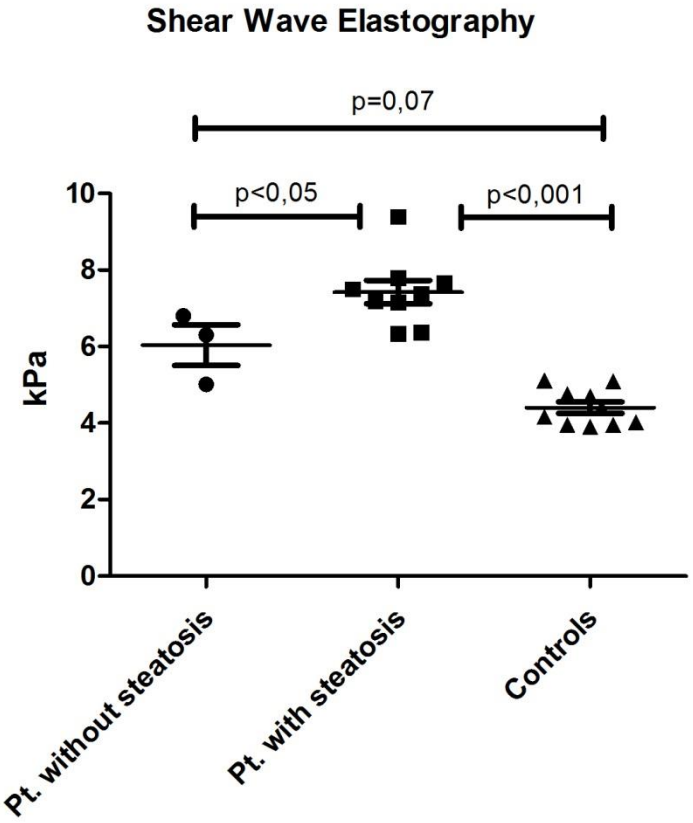
Aims: To measure the liver stiffness with SWE in severe obese patients and evaluate its relationships with the presence of steatosis.

Material and Methods: 12 patients (M/F = 1/11) aged 47± 9 yrs. with severe obesity (BMI = 43 ± 8 kg/m^2) candidated to bariatric surgery and 10 controls (M/F = 5/5, aged 46.7 ± 14.7 yrs) were evaluated after overnight fast. In all patients the presence of cirrhosis was excluded according to clinical, biochemical and imaging criteria. Patients and controls were evaluated using an Aplio i800 ultrasound system (Toshiba Medical Systems), assessing presence and grade of steatosis, left liver lobe diameter, spleen diameter and hepatic stiffness measured by SWE in the right liver lobe.

Results: Abdominal ultrasound and SWE elastography were adequately performed in all obese patients.
Hepatic steatosis was observed in 9 patients (75%), (grade I 3 p., grade II 4 p., grade III 2 p.). Hepatomegaly was observed in 8 patients (67%) and splenomegaly in 5 (42%). Liver stiffness assessed by SWE was significantly higher in obese patients than in controls (7,08±1,07 kPa vs 4,42±0,47 kPa, p<0,0001), and higher in the presence than in the absence of steatosis (7,42±0,9 kPa vs 6,03±0,9 kPa, p<0,05). A tendency to a higher liver stiffness was observed in obese patients without steatosis compared to controls but the result was not statistically significant (p=0.07).
No significant correlation was observed between liver stiffness and the grade of steatosis, or the presence of hepato- or splenomegaly.

Conclusions: Our study shows that SWE is applicable even in patients with particularly elevated BMI. Increased hepatic stiffness is present in all obese patients and particularly in those with steatosis. Further data (in progress) may help in clarifying the possible relationships with the presence and severity of steatosis and their pathophysiological mechanisms.

Figure:



Disclosure of Interest: None Declared

P09-09YI

Comorbidity specific augmented hepatic injury in a murine model of obesity-induced NAFLD and peritoneal sepsis

Paul Horn[*1,2], Sebastian Vlaic[3], Ricardo Steidl[4], Markus Bläss[4,5], Ralf A. Claus[1,4]

[1]Center for Sepsis Control and Care, [2]Department of Internal Medicine IV (Gastroenterology, Hepatology, Infectious Diseases), Jena University Hospital, [3]Research Group Systems Biology/Bioinformatics, Leibniz Institute for Natural Product Research and Infection Biology (Hans Knöll Institute), [4]Department of Anesthesiology and Intensive Care Medicine, Jena University Hospital, Jena, [5]Medical and Life Sciences Faculty, Furtwangen University, Villingen-Schwenningen, Germany

Corresponding author's email: Paul.horn@med.uni-jena.de

Introduction: Obesity and non-alcoholic fatty liver disease (NAFLD) are increasingly prevalent in the general population and may have a major impact on development and resolution of sepsis-induced hepatic organ dysfunction. Though increased sepsis-induced mortality has been shown in animal models of obesity, underlying mechanisms and specific influence of NAFLD on liver dysfunction in sepsis have not been studied yet.

Aims: To implement a murine comorbidity model of obesity-induced NAFLD and polymicrobial sepsis to study comorbidity specific signaling pathways.

Material and Methods: Six weeks old male C57BL/6 mice were fed with control diet (CD) or high fat diet (HFD) for twelve weeks to induce a metabolic-syndrome (MeS)-like phenotype. Mice were repeatedly weighted and intraperitoneal glucose tolerance test was performed. Sepsis was induced by peritoneal contamination and infection (PCI). At baseline, six and 24 hours after sepsis induction, blood and liver samples were collected. We measured laboratory markers of organ function and metabolism, performed H&E liver histology and real-time quantitative PCR (RTqPCR). Using microarray transcriptome data, we performed gene enrichment analyses of differentially expressed genes.

Results: By HFD we induced a MeS-like phenotype, constituting increased body weight, impaired glucose tolerance and hypercholesterolemia. This was accompanied by severe liver steatosis without alterations in ALT or bilirubin levels at baseline. Peritoneal sepsis lead to a significantly increased 72h mortality in the HFD group (93 % vs 47 %, p=0.022). We observed increased levels of ALT and bilirubin 24h after sepsis induction in HFD, indicating increased sepsis-induced hepatic injury. In contrast, renal function was not altered in HFD compared to CD mice 24h after sepsis induction. Gene enrichment analysis of differentially expressed genes associated with HFD-specific impact on sepsis revealed comorbidity specific regulation predominantly in metabolic pathways besides regulation of inflammatory processes. Compared to CD, we observed a more sustained effect of HFD on sepsis-induced downregulation of hepatic Cyp1a1 and Mrp2 mRNA in confirmatory RTqPCR, pointing towards a more reduced drug metabolism capacity in NAFLD comorbidity.

Conclusions: We evaluated a comorbidity model of obesity-induced NAFLD and peritoneal sepsis reflecting higher mortality rate and increased severity of sepsis-induced hepatic injury. It may serve to discover comorbidity-specific signaling pathways and new therapeutic targets.

Disclosure of Interest: None Declared

P09-10YI

miR-21 ablation prevents NASH-associated hepatocellular carcinoma

Pedro M. Rodrigues[*1], Marta B. Afonso[1], André L. Simão[1], Maria M. Gaspar[1], Cecília M. P. Rodrigues[1], Rui E. Castro[1]

[1]Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Corresponding author's email: pmvrsrodrigues@ff.ulisboa.pt

Introduction: The molecular mechanisms regulating the transition from non-alcoholic steatohepatitis (NASH) to hepatocellular carcinoma (HCC) are not entirely known. We have recently shown that concomitant miR-21 ablation and FXR activation prevents NASH development in mice.

Aims: We aimed to evaluate the role of the miR-21/PPARa pathway in NASH-associated carcinogenesis.

Material and Methods: Wild-type (WT) and miR-21 KO C57BL/6N mice were fed either a choline-sufficient, amino acid-defined control diet (CSAA; n=28) or a choline-deficient, amino acid-defined diet (CDAA; n=28) for 32 and 66 weeks. After sacrifice, serum was collected and the number of pre-neoplastic nodules counted. Liver samples were processed for histological analysis and measurement of miR-21, PPARα and metabolic relevant genes, and pro-inflammatory/pro-fibrogenic cytokines by qPCR and immunoblotting.

Results: WT mice fed the CDAA diet for 32 weeks developed macrovesicular steatosis, hepatocyte ballooning, NASH and fibrosis, concomitantly with accumulation of perivascular lymphoid cells and macrophage agglomerates. After 66 weeks, all mice on the CDAA diet developed at least one preneoplastic nodule - average of 5.2 nodules/animal - with one animal developing trabecular HCC. In addition, livers presented hyperplastic foci and anisokaryosis, as well as phenotypically altered hepatocytes. Further, hepatocytes from CDAA-fed mice were highly proliferative, as evidenced by Ki-67 staining, and expressed significantly increased levels of pro-inflammatory/fibrogenic markers, particularlly in pre-neoplastic liver tissue. miR-21 expression was significantly increased in CDAA-fed mice and further increased in HCC, concomitantly with decreased expression of PPARα and its direct transcriptional genes. Of note, miR-21 KO mice fed the CDAA diet for 32 weeks displayed markedly decreased triglyceride and fatty acid serum levels, compared with WT mice; after 66 weeks, serum transaminase levels were similar to control animals, liver nodules greatly decreased - average of 2.3 nodules/animal – and the pro-inflammatory/fibrogenic milleau reversed to almost baseline.
Conclusions: Overall, the miR-21/PPARα pathway appears to contribute to NASH-associated carcinogenesis, with its inhibiton halting HCC development. Targeting miR-21 and/or PPARα may constitute an appealing therapeutic approach to prevent NASH and its complications. (Funding: PTDC/BIM-MEC/0895/2014, SAICTPAC/0019/2015, SFRH/BD/88212/2012).

Disclosure of Interest: None Declared

P10-01YI

Mitochondrial dysfunction causes liver steatosis with a different cellular defective phenotype in DHT induced PCOS-like female rodents

Peng Cui*[1], Yi Feng[1]

[1]Department of Integrative Medicine and Neurobiology, School of Basic Medical Sciences, Shanghai Medical College, Fudan University, Shanghai, China

Corresponding author's email: 13211010081@fudan.edu.cn

Introduction: Women with polycystic ovary syndrome (PCOS) are at high risk for nonalcoholic fatty liver disease (NAFLD). It is increasingly urgent that liver cell fat accumulation and different degrees of inflammation are present in more and more PCOS women. Mitochondria is associated with lipids metabolism and cell functions such as autophagy and apoptosis.

Aims: To investigate the molecular mechanisms of mitochondrial dysfunction and subsequent changes in the development of liver steatosis in dihydrotestosterone (DHT) induced PCOS rats and mice.

Material and Methods: Materials and Methods
Animals and experimental design
21 day female wistar rats (control, DHT, n=5/group) and C57BL/6 mice (control, DHT, n=7/group) were implanted 7.5mg and 2.5mg dihydrotestosterone (DHT) tube for three months under their necks, respectively.
Histological analysis: hematoxylin and eosin (H&E) staing
Quantitative real time polymerase chain reaction (qRT-PCR) analysis
Western blot analysis
Malondialdehyde (MDA), xanthine oxidase (XOD) test

Results:

Results 1
Hepatic steatosis and different degrees of inflammation are observed on DHT treated rats and mice.

Results 2
Rats and mice treated with DHT show mitochondrial dysfunction which indicated by expression changes of protein complexes in electron transfer chain and subsequent reactive oxygen species (ROS) abnormal production.

Results 3
Apoptosis in liver cells is up regulated in both DHT treated rats and mice.

Results 4
Autophagy does not show obvious changes in rats and mice treated with DHT.

Conclusions: Our research suggests that mitochondrial dysfunction plays some role in the development of liver steatosis via driving the production of ROS and subsequent imbalance between apoptosis and autophagy in liver cells

Figure:

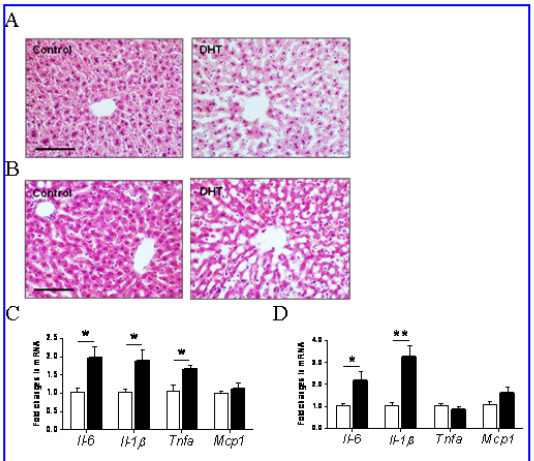


Figure 1
Liver morphology and the expression of inflammation marker in DHT treated rats and mice. (A) liver H&E staining of control and DHT rats (n=5/group), (B) liver H&E staining of mice (n=7/group). mRNA levels of hepatic inflammatory factors were determined by qRT-PCR in rats (C) and mice (D). Datas were shown as mean ± SEM, *p<0.05, **p<0.01.

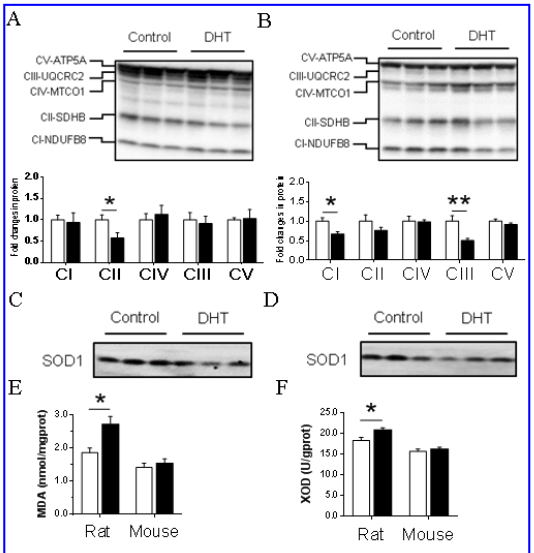


Figure 2
Expression of protein complexes in mitochondrial electron transfer chain and key enzymes associated with the production of reactive oxygen species in DHT treated rats and mice. Protein complexes changes in rats (A) and mice (B) were determined by western blot analysis, total protein served as loading control. Superoxide dismutase (SOD) of rats (C) and mice (D) were analyzed by WB, Malondialdehyde (MDA) and xanthine oxidase (XOD) in both rats and mice (E,F) were measured by kits.

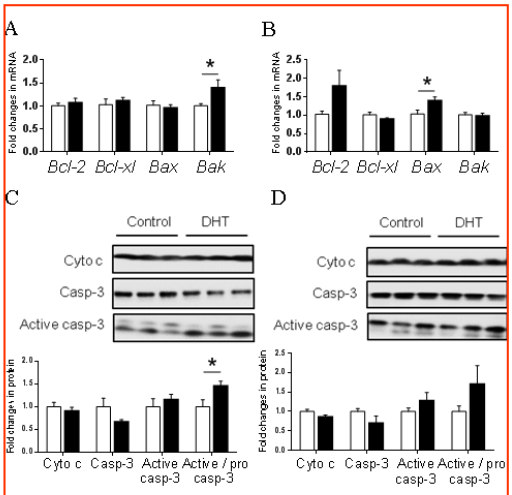


Figure 3
Changes of proapoptotic and antiapoptotic markers in DHT treated rats and mice. mRNA expression in rats (A) and mice (B) were determined by qRT-PCR, expression of apoptosis associated proteins in Rats (C) and mice (D) were analyzed by WB. Cyto, cytochrome c, casp-3, caspase-3, active casp-3, active caspase-3.

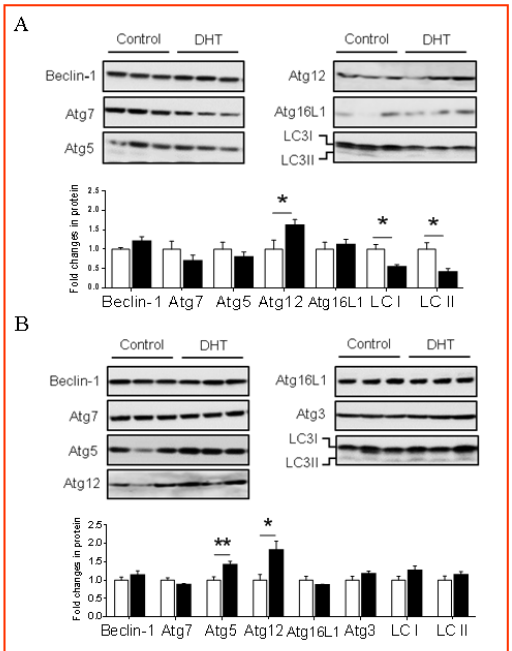


Figure 4
Expression of autophagy markers in rats and mice livers. Protein changes in rats (A) and mice (B) were analyzed by WB, total protein served as loading control, datas were shown as mean ± SEM, *p<0.05, **p<0.01.

Disclosure of Interest: None Declared

P10-02

Metabolomic profile predicts the onset of fatty liver in young adults

Peter Würtz[*1,2]

[1]*University of Helsinki, Helsinki,* [2]*Nightingale Health Ltd, Vantaa, Finland*

Corresponding author's email: sebastian.soidinsalo@nightingalehealth.com

Introduction: Non-alcoholic fatty liver is linked with numerous metabolic disturbances, however the metabolic changes preceding onset of fatty liver disease are poorly understood. High-throughput metabolomics can elucidate the metabolic aberrations related to fatty liver to clarify the pathogenesis and yield blood biomarkers for prioritising patients for further diagnostic procedures. This further opens opportunities for patient stratification and companion diagnostics

Aims: We performed detailed metabolic profiling to evaluate how blood metabolites (including lipoprotein composition, fatty acids, amino acids, and glycolysis-related metabolites) can predict the presence of and future risk for fatty liver in young adults.

Results: Metabolites across multiple pathways were strongly associated with the presence of fatty liver ($P < 0.0007$ for 60 measures in cross-sectional analyses). The strongest direct associations were observed for extremely large VLDL triglycerides (odds ratio = 4.86 per 1-SD, 95% confidence interval 3.48-6.78), other VLDL lipids, and branched-chain amino acids (e.g. leucine OR = 2.94, 2.51-3.44). Strong inverse associations were observed for HDL measures (HDL size (OR = 0.36, 0.30-0.42) and several fatty acids including omega-6 (OR = 0.37, 0.32-0.42). The biomarker associations were attenuated but remained significant after adjustment for waist, physical activity, alcohol, and smoking ($P < 0.0007$). Similar metabolic disturbances were observed already 10 years before fatty liver diagnosis, indicating multiple novel blood biomarkers for risk of fatty liver onset assessed already in young adults.

Conclusions: NMR metabolomics provides quantification of numerous blood biomarkers (lipoprotein lipids, fatty acids, and amino acids strongly predictive of the presence of fatty liver in young adults. These metabolic aberrations precede the development of fatty liver, suggesting utility for case enrichment in trials of novel therapies combating fatty liver.

Disclosure of Interest: P. Würtz: Stockholder: Nightingale Health Ltd

P10-03

Evaluation of the Epigallocatechin gallate (green tea) efficacy on the serum levels of hepatic transaminases among patients with non-alcoholic fatty liver disease: a randomized single blind clinical trial

Pezhman Alavinejad[*] [1], Narjes Zaeemzadeh[1], Seyed Ali Mard[1], Zahra pourmousa[1], Farnaz Farsi[2], Eskandar Hajiani[1], Farzad Jassemi Zergani[1] and Ahwaz GI group

[1]GI Department, Ahvaz Jundi Shapur University of medical sciences, Ahvaz, [2]Colorectal research center, Iran University of medical sciences and health services, Tehran, Iran

Corresponding author's email: pezhmanalavinejad@gmail.com

Introduction: Nonalcoholic fatty liver (NAFLD) is one of the most common liver disorders worldwide which could result in liver cirrhosis and its complication.

Aims: This randomized clinical trial is designed to evaluate the efficacy of Epigallocatechin gallate (EGCG, green tea) on the serum levels of liver transaminases and inflammatory markers among NAFLD patients.

Material and Methods: In this clinical trial, patients who diagnosed with NAFLD during 3months period included and randomly divided into 2 groups: group A (intervention group) who received 390mg of green tea extract for 3 months and group B who received placebo as control group. The serum levels of liver transaminases, blood sugar, serum TG and cholesterol and inflammatory markers measured before and after intervention and the results of collected DATA compared between2 groups.

Results: Overall 58 patients included (30 patients in group A and 28 cases as control group). At the end of the study, the green tea group showed a significant reduction in liver enzymes (aspartate aminotransferase (before45.76 ± 18.63, after 33.79 ± 12.27, P<0.001) and alanine aminotransferase (before 77.1 ± 35.87, after 53.8 ± 18.26, P <0.001)) compared with the placebo group. The serum levels of triglycerides, total cholesterol and LDL had also a decrease among intervention group as compared to baseline while these changes were not significant in comparison with placebo group (P = 0.75, 0.366 and 0.253 respectively). In addition, no significant changes occurred in sonographic grades of NAFLD among two groups.

Conclusions: green tea has a positive effect of the serum levels of liver transaminases among NAFLD and could be a therapeutic approach or recommendable supplement for this group of patients.

Disclosure of Interest: None Declared

P10-04

Algorithm to identify non-alcoholic steatohepatitis (NASH) patients with a NAS≥4 and F≥2: algorithm derived in an American screening cohort and validation in a British non-alcoholic fatty liver disease (NAFLD) cohort

Stephen A. Harrison[1], Angelo H. Paredes[2], Peter J. Eddowes[3], Michael Allison[4], Emmanuel Tsochatzis[5], Quentin M. Anstee[6], David Sheridan[7], Indra N. Guha[8], Jeremy F. Cobbold[9], Jennifer Whitehead[2], Valérie Paradis[10], Pierre Bedossa[10], Philip N. Newsome[*3]

[1]Radcliffe department of medicine, University of Oxford, Oxford, United Kingdom, [2]Brooke army medical center , Fort Sam Houston, Texas, United States, [3]NIHR liver biomedical research unit and centre for liver research , University of Birmingham, Birmingham, [4]Department of medicine, Cambridge biomedical research center, Cambridge university hospitals NHS foundation trust, Cambridge, [5]UCL institute for liver and digestive health, Royal free hospital and UCL, London, [6]Institute of cellular medicine - faculty of medical sciences, Newcastle university, Newcastle upon Tyne, [7]Institute of translational and stratified medicine, Plymouth university, Plymouth, [8]NIHR Nottingham digestive diseases biomedical research unit , University of Nottingham and Nottingham university hospitals, Nottingham, [9]Translational gastroenterology unit, Oxford university hospitals NHS foundation trus, John Radcliffe hospital, Oxford, United Kingdom, [10]Pathology department , Hôpital Beaujon, APHP, Clichy, France

Corresponding author's email: magali.sasso@echosens.com

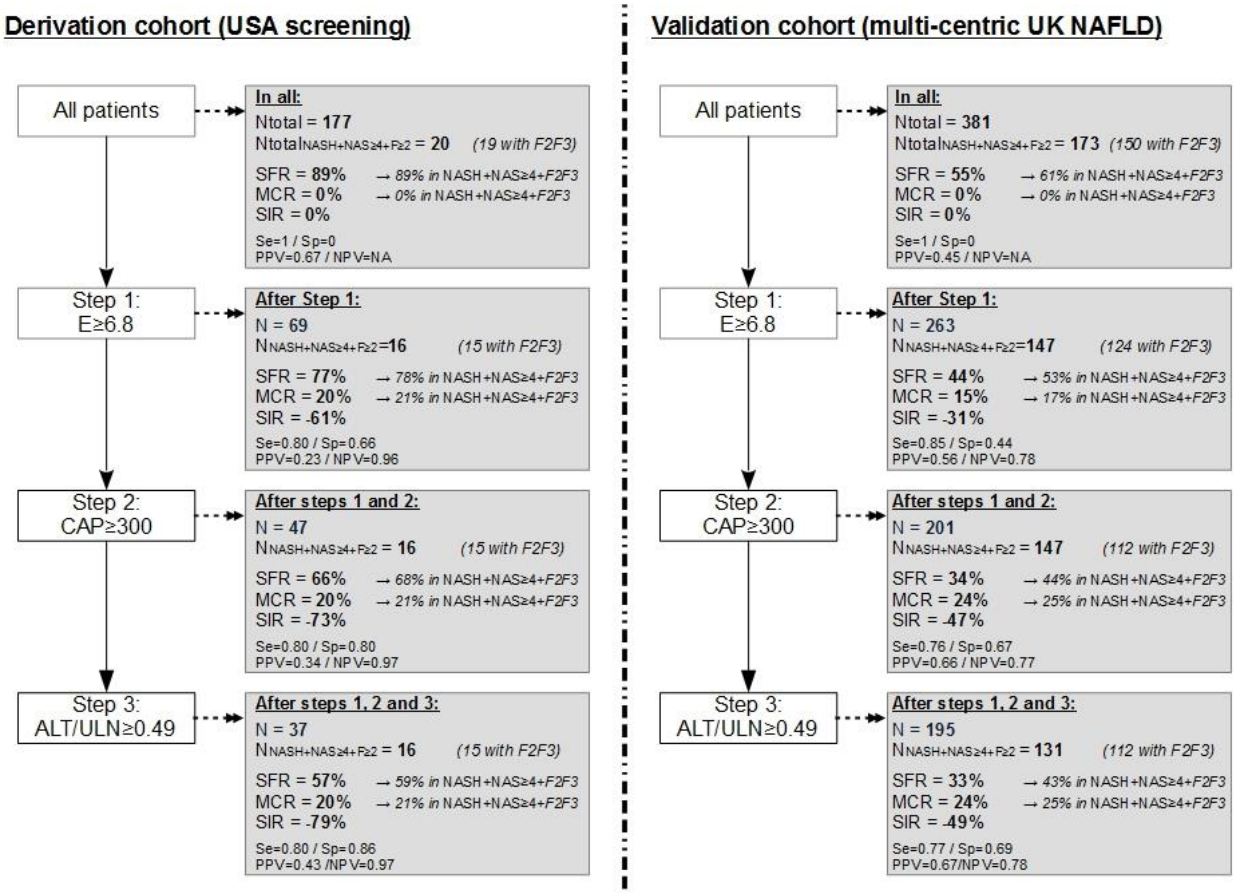
Aims: To address high screen failure rates issue in phase 2/3 clinical trials, we aimed to develop a simple algorithm to improve detection of patients with NASH that have a NAFLD activity score (NAS)≥4 and fibrosis (F) stage≥2.

Material and Methods: *Derivation cohort:* Patients referred for colon cancer screening in one American center were screened for NAFLD using FibroScan, LiverMultiScan and magnetic resonance elastography (MRE). Patients with MRI proton density fat fraction≥5% or liver inflammation and fibrosis score≥2 or E≥7kPa on FibroScan or ≥3kPa on MRE were recommended for a liver biopsy (LB). *Validation cohort:* Patients with suspected NAFLD prospectively underwent FibroScan and LB at 7 British centers. LB were read in a blinded manner with consensus by the two same expert pathologists using the NASH CRN scoring system. NASH was diagnosed using the FLIP algorithm.
The algorithm was developed to identify patients with NASH+NAS≥4+F≥2 (named "target patients") whilst keeping the level of missed cases below 25%.
The following steps were repeated 3 times:
1) Univariable analysis between the "target patients" and the bio-clinical parameters (E, CAP (controlled attenuation parameter), Fib-4, NAFLD fibrosis score, BMI, age, liver enzymes, fasting glucose, lipid parameters, platelet, albumin and ferritin),
2) Parameters significantly linked to the "target patients" were compared to select the one with the best diagnostic performance,
3) Optimal cutoff of the best parameter was computed based on a high sensitivity,
4) Patients below the cutoff were excluded.
The screen failure rate (proportion of "target patients") and missed cases rate (remaining "target patients" / initial "target patients") were calculated.

Results: *Derivation cohort:* 177 patients underwent LB. Median BMI was 32.5 [IQR=6.0] kg/m^2, age 55 [10] years. 37% were female. 39% had NASH. 11% had NASH (NAS≥4+F≥2).
Validation cohort: 381 patients underwent LB. Median BMI was 33.8 [9.3] kg/m^2, age 54 [18] years. 45% were female. 64% had NASH. 45% had NASH (NAS≥4+F≥2).
An algorithm was devised with the 3 optimally determined parameters E, CAP and ALT normalized by the upper limits of normal. Its performance is presented in the Figure.

Conclusions: A simple algorithm based on FibroScan E, CAP and normalized ALT level was developed to improve detection of NASH (NAS≥4+F≥2). If applied as a pre-screening tool in NAFLD clinical trials, it could improve the screen failure rate and reduce the number of LB with proportion of missed cases <25%.

Figure:



Legend: *SFR: screen failure rate ($N_{NASH+NAS\geq4+F\geq2}/N*100$)*
MCR: missed cases rate ($N_{NASH+NAS\geq4+F\geq2}/Ntotal_{NASH+NAS\geq4+F\geq2}*100$)
*SIR: screening improvement rate (N/Ntotal*100)*
Se: sensitivity / Sp: specificity
PPV-NPV: positive and negative predictive values
ULN: upper limit of normal

Disclosure of Interest: S. Harrison: Consultant: Merck, Perspectum, Fibrogen, Octeta, Galmed, Intercept, Genfit, NGM Biopharmaceuticals, Pfizer, the Chronic Liver Disease Foundation, BMS, Medivation, Sponsored lectures (National or International): Merck, Gilead, Abbvie, Alexion , A. Paredes: : None Declared, P. Eddowes: : None Declared, M. Allison: Grant: Evelyn Trust Grant, EU H2020 Grant Co-ApplicantEvelyn Trust Grant, EU H2020 Grant Co-Applicant, Consultant: Norgine UK Advisory Board, E. Tsochatzis: Other: Astra Zeneca, ViiV Healthcare, Q. Anstee: : None Declared, D. Sheridan: Sponsored lectures (National or International): Intercept, Gilead, Abbvie, Alexion , I. Guha: :

None Declared, J. Cobbold: Consultant: Alnylam pharmaceuticals, Sponsored lectures (National or International): BMS, Other: Abbvie, J. Whitehead: : None Declared, V. Paradis: : None Declared, P. Bedossa: Consultant: Echosens, Genfit, Intercept, P. Newsome: Consultant: AbbVie, Boehringer Ingelheim, DS BioPharma, Janssen, Norgine, Novo Nordisk, Pfizer, Shire

P10-05

Expression profiling of 728 miRNAs in a NASH model identifies excellent correlations of hepatic and circulating miR-34a levels with histological lesions in rats and men

Geneviève Cordonnier[1], Frederic Texier[1], Benoit Noel[1], Nathalie Degallaix[1], Fouad Ben Sudrik[1], John Brozek[1], Rémy Hanf[*1]

[1]Genfit SA, Loos, France

Corresponding author's email: remy.hanf@genfit.com

Introduction: Changes in the hepatic expression of miRNAs might be instrumental in the development of non-alcoholic steatohepatitis (NASH) and fibrosis.
Aims: In order to identify miRNAs for non-invasive diagnosis of NASH, the aim of this animal study was to screen for miRNAs showing good correlations of both hepatic and circulating levels with histological lesions. Suitable miRNAs were tested for their clinical utility as new circulating biomarkers of NASH in a cohort of NAFLD patients.

Material and Methods: Wistar rats were fed a control diet (CSAA), or a choline deficient amino acid diet (CDAA) with or without 1% cholesterol for 11 weeks. Hepatic expression of 728 mature rno-miRNAs was analyzed using Affymetrix miRNA chip V4.0. The most differentially expressed miRNAs (CDAA+chol vs Control) were quantified by RT-qPCR in liver and plasma samples to assess correlations with NASH histological scores and fibrosis. Diagnostic performances of circulating levels of selected miRNAs were then compared in a cohort of NAFLD patients (GOLDEN study; N=269).

Results: Expression array analysis of rat liver extracts revealed that 97 miRNAs were upregulated and 27 were downregulated in diseased livers (CDAA+chol vs control, FC≥1.5, P ≤ 0.05). The two most upregulated miRNAs were miR-132-3p (51.7 fold, p<0.0001), miR-34a-5p (26.2 fold, p<0.001), while miR-122-5p expression was not affected. RT-qPCR analyses showed that i) both hepatic and plasma levels of miR-34a strongly increased with NASH histological scores (NAS, steatosis, lobular inflammation, hepatocyte ballooning) and fibrosis stage, ii) for miR-122-5p, there was a weak negative correlation between hepatic levels and histological scores but, in contrast, there was a strong positive correlation when considering plasma levels. In NAFLD patients, serum level of miR-34a-5p increased with NAS and fibrosis stage whereas serum level of miR-122-5p increased with NAS, but not with fibrosis stage. Finally, when measuring serum levels, miR-34a-5p was more potent than miR-122-5p and miR132-3p in detection of patients with active disease and significant fibrosis (NAS≥4 and F≥2): AUROC=0.74 vs 0.59 vs 0.58.

Conclusions: Hepatic miRNA expression is profoundly modified in NASH, supporting a major role in NASH and liver fibrosis. Compared to other miRNAs and notably miR-122-5p, both hepatic and circulating levels of miR-34a are significantly correlated with the hepatic lesions, making serum miR-34a a potent biomarker with clinical utility for non-invasive diagnosis of NASH.

Disclosure of Interest: None Declared

P10-06

Drug repurposing screen to uncover compounds with antifibrotic properties: identification of phase-2 ready candidates that synergize in vitro and in vivo to treat hepatic fibrosis

Robert Walczak[*1], Carole Belanger[1], Corinne Foucart[1], Philippe Delataille[1], Sophie Mégnien[2], Dean W. Hum[1], Bart Staels[3], Scott L. Friedman[4]

[1]Drug Research, Genfit SA, Loos, France, [2]Drug Development, Genfit SA, Boston, United States, [3]UMR1011, Lille University, Lille, France, [4]Division of Liver Diseases, Icahn School of Medicine, New York, United States

Corresponding author's email: robert.walczak@genfit.com

Introduction: Fibrosis is the common end stage of chronic liver disease and is directly predictive of morbidity and mortality. Therapies to stop hepatic fibrosis progression are urgently needed and drug combinations that improve the response rate to medication are becoming a rule of thumb in chronic liver diseases, including NASH.

Aims: We have recently identified several potential antifibrotic therapy candidates based on a drug repurposing screen in a library of several hundreds of FDA approved drugs. Among those, two drugs with good efficacy and confirmed safety profile in man were selected for further investigation. The aim of the current study is to evaluate their efficacy on fibrosis *in vitro* and *in vivo*, both as single agents and in combination.

Material and Methods: Activated primary human hepatic stellate cells (HSCs) are considered as a good *in-vitro* model of myofibroblasts that mimic early stages of fibrotic response. Increase in alpha Smooth Muscle Actin protein level (αSMA), in response to TGFβ1, was used as readout. Antifibrotic efficacy was validated *in vivo,* using the CCl_4 model.

Results: Both drugs inhibited TGFβ1-induced αSMA induction in HSCs with efficacy of 80-90%, when tested as single agents. This effect was also confirmed on pulmonary and cardiac fibroblasts. When tested at suboptimal therapeutic doses, the efficacy of both drugs to inhibit αSMA in HSCs was increased by 2-3 fold if used in combination. The synergy between candidates was also confirmed *in vivo*, in the CCl4-induced fibrosis model, where fibrosis area was reduced twofold by the combination, as compared to any single agent at its most efficacious dose. Gene expression analysis by RNAseq revealed that the combination was more potent to interfere with CCl4-mediated induction of hepatic genes that are involved in fibrosis, HSC activation, ECM remodeling, inflammation and cell cycle, as compared to any single agent.
Conclusions: Two phase 2-ready drug candidates with potent antifibrotic properties and good safety profile in man were identified in drug repurposing screen on TGFβ1-activated primary human myofibroblasts. Both drugs synergize in HSCs and i*n vivo* to reduce liver fibrosis.

Disclosure of Interest: R. Walczak: Stockholder: Genfit SA, Employee: Genfit SA, C. Belanger: Stockholder: Genfit SA, Employee: Genfit SA, C. Foucart: Stockholder: Genfit SA, Employee: Genfit SA, P. Delataille: Stockholder: Genfit SA, Employee: Genfit SA, S. Mégnien: Stockholder: Genfit SA, Employee: Genfit SA, D. Hum: Stockholder: Genfit SA, Employee: Genfit SA, B. Staels: Consultant: Genfit SA, S. Friedman: Consultant: Genfit SA

P10-07YI

Modelling PNPLA3-induced non-alcoholic fatty liver disease using human pluripotent stem cells

Samantha G. Tilson[*1, 2, 3], Carola M. Morell[2], Casey A. Rimland[2], An-Sofie Lenaerts[2], Jake Liang[3], Ludovic Vallier[1, 2]

[1]Wellcome Trust Sanger Institute, [2]Wellcome Trust-Medical Research Council Stem Cell Institute, University of Cambridge, Cambridge, United Kingdom, [3]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, United States

Corresponding author's email: samanthagtilson@gmail.com

Introduction: Non-alcoholic fatty liver disease (NAFLD) affects one-third of adults in developed countries. This disease is characterized by the accumulation of fat within the liver that can lead to inflammation, fibrosis, and hepatocellular carcinoma. Until recently, NAFLD has been considered a largely metabolic disease; however, recent studies have suggested that genetic factors could also have major influence on disease onset and evolution. Accordingly, genome wide association studies (GWAS) have identified several genetic variants that are strongly associated with the development and progression of NAFLD regardless of underlying metabolic disease. The most commonly and significantly identified variant is the I148M variant in the gene coding for Patatin-like phospholipase domain-containing protein 3 (PNPLA3).

Aims: Although the I148M variant has been strongly correlated with NAFLD clinically, very little is known about the mechanisms by which it alters lipid metabolism and causes disease progression through fibrosis/inflammation. In order to elucidate this mechanism, we decided to take advantage of the unique proterties of human induced pluripotent stem cells (hiPSCs) and the CRISPR/CAS9 gene editing technology.

Material and Methods: We chose to use hiPSCs because these cells can be easily genetically modified while maintaining their capacity to differentiate into a diversity of cell types. Thus, we used CRISPR/CAS9 to introduce mutations of interest in the PNPLA3 gene of hiPSCs. We generated hiPSC lines with either a complete knock-out of the PNPLA3 gene or with the I148M variant knocked-in. These genetically edited cells were then differentiated into hepatocytes and treated with free fatty acids to model the NAFLD phenotype in vitro.

Results: Both the knock-out and knock-in clones showed similar differentiation capacity as wild-type cells thereby suggesting that the absence of PNPLA3 does not affect basic hepatocyte functions. When treated with fatty acids, the genetically edited cells appear to accumulate more lipid droplets indicating that mutations in PNPLA3 interfere with the lipid metabolism of hepatocytes.

Conclusions: Once fully established and characterized, these hiPSC lines will provide the first opportunity to fully analyse the role of PNPLA3 in the development and progression of NAFLD *in vitro*. Considered together, these results demonstrate that hIPSCs provide a unique platform not only to validate genetic variants involved in NAFLD but also to identify potential targets for future drug development.

Disclosure of Interest: None Declared

P10-08

Features and outcome of concomitant non-alcoholic liver disease and celiac disease in adults: A large prospective longitudinal study

Sanaa Kamal[*] [1], Sara AbdelHakam[1], Khaled Al Dossari[2], Dahlia Ghoraba[1], Amgad Hassan[1], Tamer Hafez[1]

[1]Gastroenterology, Ain Shams Faculty of Medicine, Cairo, Egypt, [2]Family Medicine, Prince Sattam University, Kharj, Saudi Arabia

Corresponding author's email: sanaakamal@ainshamsmedicine.net

Introduction: Concomitant non-alcoholic fatty liver disease (NAFLD) and celiac disease (CD) has not been adequately studied.

Aims: This study investigated the frequency of CD among NAFLD patients and the clinico-pathologic and immunologic patterns and outcome of concomitant NAFLD and CD.

Material and Methods: This prospective longitudinal study screened NAFLD patients for CD (tissue transglutaminase antibodies; anti-TTGA and antiendomysial antibodies; EMA). Patients with concomitant NAFLD and CD and patients with either NAFLD or CD were enrolled and followed. Duodenal biopsy, transient elastography (TE), TNF-alpha, TGF-beta, interleukins 1, 6, 10, 15, 17, folic acid and vitamins B12 and D were performed at baseline and one year after gluten free diet (GFD).

Results: CD was confirmed in 7.2% of NAFLD patients. Refractory anemia and nutritional deficiencies were frequent in patients with concomitant NAFLD and CD who had advanced intestinal and hepatic lesions, higher levels of TNF-a, IL-15 and 17 compared to CD and NAFLD patients. Patients with CD with NAFLD showed clinical response to GFD but intestinal histologic improvement was suboptimal. Combining EMA-IgA or anti-TTGA with either IL-15 or IL-17 enhances the prognostic performance of both tests in predicting histologic response to GFD.

Conclusions: Concomitant NAFLD and celiac disease is not uncommon. Recurrent abdominal symptoms, refractory anemia, nutritional deficiencies in NAFLD patients warrant screening for celiac disease. The study has important clinical implications since failure in diagnosing CD in NAFLD patients' results in marked intestinal and hepatic damage and sub-optimal response to GFD that can be alleviated by early diagnosis and initiation of GFD.

Disclosure of Interest: None Declared

P10-09

Optimal cut-off value to assess changes of intrahepatic fat amount using controlled attenuation parameter in longitudinal setting

Sang Bong Ahn*[1], Dae Won Jun[2], Bo-Kyeong Kang[3]

[1]Department of Internal Medicine, Eulji University School of Medicine, [2]Department of Internal Medicine, [3]Department of Radiology, Hanyang University School of Medicine, Seoul, Korea, South

Corresponding author's email: dr486@hanmail.net

Introduction: Controlled attenuation parameter (CAP) has shown a good correlation with intrahepatic fat amount in cross-sectional study. However, there is no study whether change of CAP scores can also show a good correlation in longitudinal setting. Therefore, we investigated the correlation between CAP and magnetic resonance imaging-estimated proton density fat fraction (MR PDFF) by serial examination in longitudinal setting. Controlled attenuation parameter (CAP) has shown a good correlation with intrahepatic fat amount in cross-sectional study. However, there is no study whether change of CAP scores can also show a good correlation in longitudinal setting.

Aims: We investigated the correlation between CAP and magnetic resonance imaging-estimated proton density fat fraction (MR PDFF) by serial examination in longitudinal setting.

Material and Methods: Sixty-five nonalcoholic fatty liver disease (NAFLD) patients were evaluated with MR PDFF and transient elastography including CAP at baseline and three months later.

Results: CAP and MR PDFF at baseline showed a strong correlation in assessing hepatic steatosis ($r=0.66$, $p<0.001$). After treatment, the correlation between CAP change after treatment and intra-hepatic fat change (%) using MR PDFF was not satisfactory ($r=0.37$, $p=0.005$) in longitudinal setting. The optimal cut-off value of CAP change for discriminating an improvement/aggravation in intra-hepatic fat % (more than 1% change of MR PDFF) was selected as 38dB/m (AUROC=0.559). For CAP changes greater than 38dB/m, the predictive value was 14/16 (87.5%), whereas, for CAP changes less than 38dB/m, it was 12/41 (29.3%). As the result, the accuracy of the method using CAP change was only 26/57 (46%). In addition, Cohen's kappa value was not significant ($\kappa=0.11$, $p=0.186$). Hence, we cannot conclude that CAP is a sensitive test for predicting hepatic steatosis changes.

Conclusions: CAP showed a good diagnostic ability to discriminate hepatic steatosis. A careful interpretation of the steatosis change using CAP score is needed when the absolute change value is less than 38dB/m in clinical setting.

Disclosure of Interest: None Declared

P10-10

Effectiveness of a multidisciplinary team in NAFLD patients' management

Sara Policarpo*[1], Mariana V. Machado[2], David Barreira[2], Helena Cortez-Pinto[2]

[1]Serviço de Dietética e Nutrição, [2]Serviço de Gastrenterologia, Hospital Santa Maria, Lisbon, Portugal

Corresponding author's email: saraopolicarpo@gmail.com

Introduction: Lifestyle changes are the mainstay of the management of nonalcoholic fatty liver disease (NAFLD), however they are very difficult to implement.

Aims: We aimed to assess the efficacy of a multidisciplinary team (gastroenterologist, dietitian and psychologist) in a combined intervention in patients with NAFLD.

Material and Methods: Patients with NAFLD followed, at least 3 months, by a multidisciplinary team, were included. Lifestyle modification, physical activity and the associated psychological factors and diet aiming weight loss were applied. Anthropometric and clinical data [including liver biochemistry, elastography, NAFLD Fibrosis Score (NFS) and Fibrosis 4 score (FIB4)] were collected at baseline and after 3 months. Patients previously followed in the Hepatology Unit were evaluated before and after a multidisciplinary approach.

Results: From the Hepatology ambulatory clinic, 39 out 87 patients with NAFLD accepted to be followed by the multidisciplinary team; 72% males, age 56±11 years, mean time of diagnosis 9.1±6.7 years (range 1-22 years). At baseline, BMI was 31.2±5.2 kg/m^2, with 89.7% of patients being overweight; 48.4% of patients F2-F3 and 25.6% F3-F4 as assessed by elastography; FIB4 was >2.67 in 16.7% of patients and NFS >0.675 in 8.6%. After 3 months, 94.9% of patients lost weight with 25.6% achieving a reduction in their BMI category, 92.3% reduced their waist circumference (3cm). Liver enzymes decreased in the majority of patients (AST in 80% and ALT in 84%), NFS decreased in 31.8% and FIB4 in 57.1%. No correlations were found between weight loss and markers of NAFLD activity. 25 patients were previously followed in Hepatology Unit, and only 20% had lost weight before joining the multidisciplinary team, with a global positive variation in weight (0.8±1.01%) as compared to negative variation after 3 months (4.9±0.5%, p<0.001). Similarly, previous to multidisciplinary intervention, AST only decreased in 45% (globally increased 72±35% *vs.* 19±10 decrease after 3 months, p=0.048) and ALT decreased in 38% (globally increased 86±38% *vs.* 27±11 decrease after 3 months, p=0.023).

Conclusions: In a small sample, we demonstrated that a multidisciplinary team can improve the management of patients with NAFLD.

Disclosure of Interest: None Declared

P11-01YI

Randomized placebo-controlled single blind clinical trail: The hepatoprotective effect's result of new lonal drug in patient with non-alcoholic fatty liver disease

Sarnai Tsagaankhuu[*] [1], Ariunaa Zundui[2], Jargalan Lkhagvasuren[3], Badamjaw Sewjid[4], Erdenetsogt D[5]

[1]Internal medicine, Professor's team of the hepatology, MNUMS, [2]Treatment, Research and Production Company of the Mong-Em, [3]Wuhan University of Technology, [4]internal medicine, Professors of the hepatology, [5]Department of Pathology, School of Pharmacy and Bio Medicine, MNUMS, Ulaanbaatar, Mongolia

Corresponding author's email: sarnai0904@yahoo.com

Introduction: Our studying the new Lonal drug's raw material is *Lonicera Altaica Pall* that has been using in traditional medicine for the liver disease, cholecystitis, edema, heart disease, hypertension, anemia, to improve the dysfunction of stomach and intestine, to support the important organs of life and pursue dairy drink appetite.

Aims: The aim of our clinical trial was to determine hepatoprotective effect of the new Lonal drug in patient with non alcoholic fatty liver disease (NAFLD).

Material and Methods: Research design is a *Randomized Placebo-Controlled, Single Blind Clinical Trial.*

Results: Lonal drug significant decreases hepatocellular and cholestatic injury and reduces TG ($p<0,05$), increases HDL ($p<0.047$). That was compared to determine before and after treatment such as steatosis and fibrosis degree of participants liver by fibroscan and liver biopsy. Before treatment steatosis degree was S2, after treatment it was dropped S1 ($p<0.05$). And before treatment, fibrosis degree F2-3, after treatment it was decreased F1-2 ($p<0.01$). In liver biopsy, comparing before and after treatment the results of hepatocellular inflammation and fibrosis area was reduced by 1,75 times and decreases hepatic steatosis degree (Strong fatty change was reduced mild fatty change).

Conclusions: New Lonal drug is reducing hepatocellular injury, cholestatic injury, repairing some criteria of metabolic syndrome and reducing the degree of liver steatosis and fibrosis by fibroscan and liver biopsy in patient with NAFLD.

Disclosure of Interest: None Declared

P11-02

The dual amylin and calcitonin receptor agonist, KBP-089, improves metabolic and hepatic features of nonalcoholic steatohepatitis in high fat, high cholesterol fed rats

Sofie Gydesen[*] [1], Sam J. Daniels[1], Anna T. Larsen[1], Nina Sonne [1], Morten A. Karsdal[1], Kim Henriksen[1]

[1]Nordic Bioscience, Herlev, Denmark

Corresponding author's email: sofiegydesen88@gmail.com

Introduction: Obesity and non-alcoholic fatty liver disease (NAFLD) are the most common causes of nonalcoholic steatohepatitis (NASH) and subsequently chronic liver disease, such as fibrosis. While no treatments are approved, weight loss and insulin sensitizers have shown promise on metabolic and hepatic parameters; hence, drugs causing weight loss and alleviating insulin resistance are highly interesting as candidates for treatment of NAFLD and NASH. Here we present a highly potent dual amylin- and calcitonin receptor agonist, KBP-089, and evaluate the effect on body weight, glucose control as well as different hepatic features of NASH in rats.

Aims: Here we present a highly potent dual amylin- and calcitonin receptor agonist, KBP-089, and evaluate the effect on body weight, glucose control as well as different hepatic features of NASH in rats.

Material and Methods: 6-week-old rats received a high fat diet (HFD) for 8 weeks to induce significant obesity followed by a high fat, high cholesterol and cholate diet (HFCC) for 56 days. After HFD, the rats were assigned into treatment groups receiving either vehicle (saline) or KBP-089 in four doses. All the rats were dose escalated weekly starting from the lowest dose 0.625 µg/kg and to 1.25, 2.5 and 5.0 $\mu g \cdot kg^{-1}$, respectively.

Results: 8 weeks of KBP-089 treatment significantly reduced body weight – 16.5% in the group receiving the highest dose – reduced overall adiposity, and the HFCC diet induced hepatomegaly was dose-dependently reduced; a reduction, which was equalized when, normalized to the individual body weight.
Additionally, KBP-089 treatment reduced total triglyceride and AST levels. At the histological level, the HFCC feeding induced massive lipid accumulation, ballooning and inflammation as well as mild fibrosis. Notably, after treatment with KBP-089 for 8 weeks, this inappropriate storage of lipids, ballooning, and inflammation were reduced by treatment with KBP-089, hence significantly reducing the combined NAFLD activity score. Moreover, KBP-089 significantly reduced the fibrosis stage by approximately 60%.
Finally, treatment with KBP-089 improved glucose tolerance and enhanced insulin action during and oral glucose tolerance test, resulting in significantly reduced AUC values for both glucose and insulin.

Conclusions: In conclusion, KBP-089 is a weight reducing agent, which improves insulin action and hepatic features of NASH, hence revealing the potential of KBP-089 as a novel therapeutic target for the treatment of NAFLD and NASH.

Disclosure of Interest: S. Gydesen: Employee: Nordic Bioscience, S. Daniels: Employee: Nordic Bioscience, A. Larsen: None Declared, N. Sonne: None Declared, M. Karsdal: Stockholder: Nordic Bioscience, K. Henriksen: Stockholder: Nordic Bioscience

P11-03YI

Altered gut microbiota and resulting monocytosis and immunomodulation is key to NAFLD pathogenesis: A northeast India based study

Sujoy Bose[*1], Moumita Bose[1,2], Tarun K. Basumatary[1,2], Purabi D. Bose[2], Anjan K. Saikia[3,4]

[1]Biotechnology, Gauhati University, [2]Molecular Biology and Biotechnology, Cotton University, [3]Medicinal and Gastroenterology unit, Central hospital, NF Rly, [4]Gastroenterology, Hayat hospitals, Guwahati, India

Corresponding author's email: sujoybose1@gmail.com

Introduction: Mechanism of NAFLD pathogenesis is inconclusive.

Aims: Present study focused on alterations in gut-microbiota and its clinical relevance with NAFLD susceptibility and severity in tribal cases enrolled from northeast India.

Material and Methods: Clinically characterized NAFLD cases (N=76) with clinical details and fibroscan based liver stiffness measurement(LSM) score were enrolled along with community based healthy controls (n=80). Decidual aspirations based microbiome load and diversity analysis was performed by standard culture and microbial metagenomics methods for NAFLD and representative HC cases respectively. Serum endotoxin levels were estimated using standard kit (*Lonza*). Differential sCD14, mCD14, CD40, TLR4, NK/NKT expression was analyzed by ELISA/flowcytometry. Differential cytokine profile was studied by ELISA method. HepG2 cell line based microbial stimulation studies were performed for specificity of differential monocyte and cytokine activation profile. Statistical analysis was performed by SPSSv13.0 software.

Results: Increased total and gram negative bacterial load was associated with NAFLD compared to HC. Metagenomic profiling including alpha and β-diversity data indicated a sharp difference in microbiota in NAFLD cases compared to HC. Serum endotoxin and sCD14 levels were higher in NAFLD cases. Monocyte CD14 expression was significantly higher in NAFLD cases (69.92±43.07%) compared to HC (44.30±28.84%)(p=0.019). The expression of monocyte activation marker CD40 was also significantly increased in NAFLD (p=0.043) cases. Average TLR4 expression on blood cells was also higher in NAFLD cases (17.01±9.62%) compared to HC (10.86±7.92) (p=0.071). Monocyte activation marker CD40 and TLR4 levels significantly positively correlated with higher LSM scores; while mCD14 levels also correlated positively with higher LSM score. NK cell expression was higher in NAFLD. Distinct up-regulation of NFkBp65, TNF-α, IL-12 combined with significant down-regulation of anti-inflammatory cytokine IL-10 at both protein and mRNA level was found to be significantly correlating with NAFLD pathogenesis and higher LSM score (p<0.001). The hepG2 cell line with NAFLD and HC microbial antigen stimulation and co-culture study also indicated the increased monocyte activation and inflammatory cytokine expression on stimulation with microbes of NAFLD patient's origin.

Conclusions: Altered gut microbiota and resulting altered monocytosis and hyper-immunomodulation is specific and detrimental to NAFLD susceptibility and severity.

Disclosure of Interest: None Declared

P11-04YI

Serum bile acids and fibroblast growth factor 19 levels are associated with fibrosis and hepatic inflammation in hepatocellular cancer based on NASH

Svenja Sydor*[1], Paul Manka[2], Insa Messerschmidt[2], Annemarie Wegehaupt[2], Jan Best[2], Klaas Nico Faber[3], Han Moshage[3], Marino Venerito[1], Alexander Link[1], Guido Gerken[2], Ali Canbay[1], Lars P. Bechmann[1]

[1]Department of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke-University Magdeburg, Magdeburg, [2]Department of Gastroenterology and Hepatology, University Hospital Essen, Essen, Germany, [3]Department of Gastroenterology and Hepatology, University Medical Center Groningen, Groningen, Netherlands

Corresponding author's email: svenja.sydor@med.ovgu.de

Introduction: Non-alcoholic fatty liver disease (NAFLD), which may proceed to non-alcoholic steatohepatitis (NASH), is among the most prevalent liver diseases in Western societies. Given its emerging prevalence, the incidence of NASH related hepatocellular carcinoma (HCC) is increasing dramatically. In contrast to most chronic liver diseases, HCC in NASH may occur without pre-existing cirrhosis. In previous studies we have shown that serum bile acid (BA) levels correlate with disease severity in NAFLD. BA metabolism emerged as an important signalling pathway in tumorigenesis. Fibroblast growth factor 19 (FGF19) regulates BA synthesis, can affect glucose and lipid metabolism and has recently been linked to HCC.

Aims: The aim of the present study was to compare mediators of BA signalling in serum of patients with NASH and NASH-HCC with and without cirrhosis.

Material and Methods: Here, we analysed data and serum from patients suffering from NASH without cirrhosis (NO), NASH with cirrhosis (NC), NASH-HCC without cirrhosis (HO) and NASH-HCC with cirrhosis (HC). Among others, serum BA, FGF19, cell death markers such as caspase cleaved (M30) and un-cleaved (M65) CK18, and adiponectin levels were determined.

Results: In patients with NASH (n=30) serum BA and FGF19 levels were significantly higher than in NASH-HCC (n=34), while levels of the adipocytokine adiponectin were significantly lower. Overall cell death, as assessed via M65 ELISA, beside gamma-glutamyl-transferase (GGT) was significantly higher in NASH-HCC. Comparing patients with and without cirrhosis, we performed one-way ANOVA and found that BA and FGF19 levels were significantly higher in HC vs. NO, while there was no difference in BA and FGF19 levels between NC and HO. Here, BA levels are associated with fibrosis (NAFLD fibrosis score, transient elastography, and thrombocytopenia), while FGF19 is associated with hepatic inflammation (AST, GGT) and tumour markers (AFP, DCP).

Conclusions: In patients with NASH-HCC, serum BA and FGF19 levels were significantly higher as compared to NASH. While BA levels seem to be associated with cirrhosis, FGF19 correlates with hepatocellular injury and tumour markers. Characterisation of individual BA composition as well as secondary BA and investigations concerning a potential role of the gut microbiome will be needed to deepen the impact of BA metabolism within the genesis of NASH-HCC.

Disclosure of Interest: None Declared

P11-05

Correction of intrahepatic microcirculation disorders by L-ornithine-L-aspartate at the non-alcoholic steatohepatitis patients

Tatiana Ermolova[*1]

1Nord-West State Medical University, St.-Petersburg, Russia

Corresponding author's email: t.v.ermolova@mail.ru

Introduction: The basis of initial component of increased intrahepatic vascular resistance at the chronic liver diseases are endothelial disfunction, activation of hepatic stellate cells, hyperammoniemia. In some only experimental studies in vivo and in vitro was demonstrated effect of hypoammoniemic drug L-ornithine phenylacetate for liver microcirculation due to decrease of activity of hepatic stellate cells, portal hypertension, increase of endothelial nitric oxide synthase.

Aims: Aims of our study are to estimate intrahepatic microcirculation and efficacy of hypoammoniemic L-ornithine-L-aspartate (LOLA) for correction of intrahepatic hemodinamics disorders at the non-alcoholic steatohepatitis (NASH).

Material and Methods: We investigated 78 patients with NASH, minimal fibrosis 0-1 stage. Stage of liver fibrosis was estimated by transient elastography (FibroScan). Intrahepatic hemodinamics are determined by polyhepatography - modificated hepatic impedansometry, non-invasive method (PHG). PHG registers a blood flow in projection of zone of hepatic right, left lobes and spleen, integral body impedansography. Our study manifested a high degree of sensitiveness (99%) and specificity (89%) of PHG for definition of localization of hemodynamic disorders in liver (presinusoidal, sinusoidal). For correction of blood flow disorders we used hypoammoniemic drug LOLA in dosage 5 grams 3 times daily 4 weeks. Efficacy of LOLA we looked in 2 and 4 weeks via the control PHG.

Results: Analysis of PHG demonstrated, that at all patients with NASH we revealed a liver microcirculation disorders - increased blood resistance, abnormal forms and amplitude of waves ("plateau" wave) in sinusoidal level (out flow zone).Analysis of efficacy of LOLA showed, that LOLA was effective for correction of hepatic microcirculation disorders. In 2 weeks of the treatment we observed normalization or improvement of the wave form, in 4 weeks – wave amplitude.

Conclusions: NASH is characterized by disorders of intrahepatic microcirculation on sinusoidal level even in initial stage of liver fibrosis. LOLA improved liver microcirculation at the NASH patients.

Disclosure of Interest: None Declared

P11-06YI

Anti-oxidant role in reversing Non-Alcoholic Steatohepatitis (NASH) and Hepatocellular Carcinoma (HCC)

Valentina Leone[*+1,] [2], Eleni Kotsiliti[1+], Horst Zitzelsberger[3], Kristian Unger[3], Mathias Heikenwälder[1]

[1]Department of Chronic Inflammation and Cancer, DKFZ , Heidelberg, [2]Department of Radiation Science, Helmholtz Zentrum München, Neuherberg, [3]Department of Radiation Science, Helmholtz Zentrum München, Neuhenberg, Germany
[+] Equally contributing authors

Corresponding author's email: valentina.leone88@gmail.com

Introduction: Alterations in our life-style including high caloric intake combined with a sedentary life style have augmented the incidence of obesity over the last decades, and it´s expected to increase in the near future. Liver is strongly affected by high caloric intake, leading to metabolic syndrome, steatosis and steatohepatitis. Non-alcoholic steatohepatitis (NASH) constitutes a risk factor for hepatocellular carcinoma (HCC), the most common primary liver malignancy and the third most common cause of cancer related death. It has been demonstrated in our lab that long-term choline-deficient high-fat diet (CD-HFD) induces HCC with an incidence of 25%. In HCC mouse models and in human HCC specimens high levels of ROS were found: hepatic mitochondrial dysfunction drives severe liver damage and hepatocyte proliferation constituting an environment pro-carcinogenic.

Aims: To study the role of ROS in NASH and HCC, we tested the anti-oxidant compound butylated hydroxy anisol (BHA) in combination with CD-HFD. Our aim is to assess whether therapeutic treatment with BHA –still under CD-HFD- in the presence of NASH would reduce cancer incidence or would even reverse the NASH phenotype.

Material and Methods: C57Bl/6 mice were fed long-term with different diets (normal diet,CD-HFD and BHA+CDHFD) for 12 months.Murine livers were analyzed by immunohistochemistry (IHC), flow cytometry, qRT-PCR and Western blot.Gene expression profiling was performed on Agilent platform with G3 Mouse Gene Expression 8×60k v2 microarrays.

Results: The diet-switch group (6-months CD-HFD/6-months BHA+CDHFD) revealed absence of liver damage and fibrosis, reduced weight and steatosis when compared to CD-HFD group. Inflammation was also reduced, as shown by IHC and flow cytometry analysis.The diet-switch group showed rescue from glucose impairment and absence of NASH compared with CD-HFD fed mice. Almost all mice were protected from HCC, as only one case out of 48 developed HCC after 12-months under BHA+CDHFD, differently from the mice under CD-HFD only (incidence 28%).Next we assessed whether fatty liver disease and NASH could be prevented by giving mice BHA+CDHFD prophylactically.The mice gained weight similarly to the normal diet cohort, they showed no liver damage and no glucose uptake impairment.Moreover, mice presented significantly reduced inflammation.Liver histology revealed lack of steatosis and fibrosis.

Conclusions: Taken together, the above data indicate oxidative stress to be a potential important step in NASH and subsequent HCC development.

Disclosure of Interest: None Declared

P11-07

Relations between carotid artery wall thickness and liver fibrosis in subjects with NAFLD

Varun Mehta*[1], Sahil Nohria[1]

[1]*Gastroenterology, DMC&H, ludhiana, India*

Corresponding author's email: nohriasahil@gmail.com

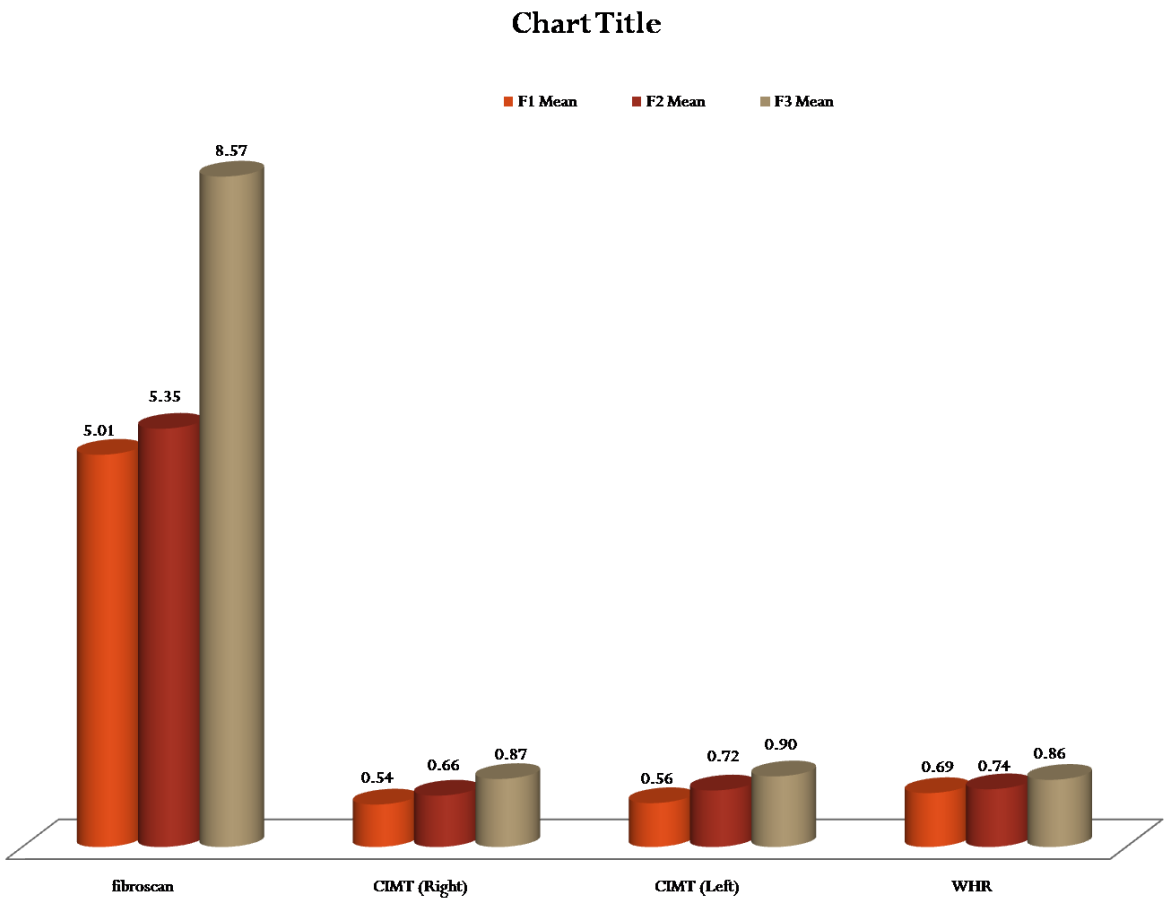
Introduction: Studies have shown that the existence of fatty liver in diabetics is associated with an increase in cardiovascular morbidity and mortality specially caused by the liver. Liver biopsy is the gold standard of liver fibrosis evaluation, but it is a painful and invasive procedure. Carotid intima-media thickness (IMT) is one of the subclinical markers of atherosclerosis. Liver stiffness (LS) measurement using transient elastography (TE) has emerged as a promising noninvasive tool for assessing the degree of liver fibrosis

Aims: This study intends to correlate the liver stiffness assessed using fibroscan with Carotid intima media thickness in NAFLD patients so that timely intervention can be done to prevent cardiac complications.

Material and Methods: All NAFLD patients who underwent Fibroscan and agreed for Carotid Intima Media Thickness (CIMT) were recruited in this study. Diagnosis of fatty liver was based on abdominal ultrasonography. History and physical examination was recorded. Lab investigations included complete blood count, LFT, RFT, glycosylated hemoglobin, lipid profile, fasting insulin level, fasting blood sugar levels.

Results: A total of 55 patients who had NAFLD were studied. Patients were divided into 3 groups according to grades of fatty liver. Patients with grade III fatty liver on ultrasound were found to have markedly greater CIMT values and fibrosis (p value <0.001) as compared to grade I and grade II fatty liver. Also BMI, WHR, dyslipidemia, IFG was significantly higher in subjects with grade III fatty liver.
Conclusions: Individuals with higher grade of fibrosis are strongly associated with increased carotid intima thickness along with increased risk of metabolic syndrome

Figure:



Disclosure of Interest: None Declared

P11-08YI

Fatty liver is associated with increased visceral and cardiac fat and cardio metabolic risk

Veronica Della Latta*[1], Fabrizia Carli[1], Melania Gaggini[1], Emma Buzzigoli[1], Anna Maria Sironi[1], Amalia Gastaldelli[1]

[1]*institute of clinical physiology CNR - Pisa, Pisa, Italy*

Corresponding author's email: vdellalatta@ifc.cnr.it

Introduction: NAFLD is associated to metabolic syndrome and is a major risk factors for cardio-metabolic diseases.

Aims: The purpose of this study was to evaluate if presence of fatty liver was associated to increased accumulation of visceral and epicardial fat and if fatty liver index (FLI) might be used as a marker of increased cardio-metabolic risk.

Material and Methods: We studied 113 subjects at risk of metabolic diseases. Subjects were screened for glucose tolerance by OGTT; fat amount was evaluated by bioimpedence and distribution in abdominal (visceral, VF; subcutaneous, SC) and cardiac (epicardial, EPI; extrapericardial, PERI) fat depots by Magnetic Resonance Imaging (MRI). NAFLD was evaluated using FLI (FLI >60) and was confirmed by Magnetic Resonance Spettroscopy (MRS) in 37 subjects. We examined the association among FLI and abdominal (visceral and subcutaneous), cardiac (epicardial and extrapericardial) and hepatic fat and with factors of metabolic syndrome.

Results: Subjects were divided into three groups: FLI<20 (n=20); FLI 21-59 (n=50); FLI >60 (n=25). FLI was increased proportionally to total, abdominal and cardiac fat. Subjects with FLI>60 have the largest fat depots, abdominal and cardiac (mainly VF and PERI), ($p<0.001$ vs FLI<20). FLI, VF and PERI were all correlated with factors of metabolic syndrome, in particular BP and TG.

Conclusions: FLI is a good marker of increased cardio metabolic risk and of ectopic fat accumulation not only as hepatic fat but also as visceral and cardiac fat.

Disclosure of Interest: None Declared

P11-09YI

Role of gender in juvenile Non-Alcoholic Fatty Liver Disease (NAFLD) pathogenesis

Veronica Marin*[1], Silvia Gazzin[1], Alan Raseni[2], Claudio Avellini[3], Claudio Tiribelli[1], Natalia Rosso[1]

[1]Italian Liver Foundation, [2]S.C. Laboratorio Analisi Cliniche, IRCCS Burlo Garofalo, Trieste, [3]Dip. Anatomia e Patologia, Azienda Ospedaliero-Universitaria "Santa Maria della Misericordia", Udine, Italy

Corresponding author's email: veronica.marin@fegato.it

Introduction: NAFLD is more common among males, with a boom in prevalence in middle-aged women.

Aims: This study aimed to assess the events involved in the progression of the disease in both genders.

Material and Methods: Male and female C57BL/6J mice were fed after weaning with control diet (CTRL) or high-fat high-carbohydrate (HFHCD) for 16 weeks; thereafter, biochemical and biomolecular analysis have been performed. Data was compared *vs* animals of each gender fed with CTRL diet.

Results: Males and females fed with HFHCD showed a significant weight gain, adipose tissue hypertrophy, hepatomegaly, hyperlipidemia, increased ALT and alterations in glycemia. Hyperinsulinaemia was present exclusively in males. Histological analysis showed hepatic steatosis in both genders, in line with the increased expression of DGAT2 (important in the final step of triglycerides synthesis), LDL receptor and SREBP-1c (involved in the lipogenesis). Likewise, both groups developed sinusoidal/periportal fibrosis with increased activation of hepatic stellate cells (α-SMA) and collagen deposition (confirmed at mRNA and protein level). Surprisingly, only males showed apoptosis, inflammatory foci and increased expression of TNF-α, whereas females presented increased lipid peroxidation, oxidative stress (absent in males) with no signs of inflammation. Leaky gut was also displayed in both genders with a marked reduction of Claudin-5 and ZO-1 protein expression in the large intestine.

Conclusions: Altogether, these data suggest that even if the presence of fibrosis is similar between genders, the pathogenesis is different: in males is associated with apoptosis, insulin resistance and inflammation whereas in females is mostly related to oxidative process.

Disclosure of Interest: None Declared

P11-10

Pharmacologic inhibition of hepatic acetyl-CoA carboxylase activity produces improvements multiple dimensions of NASH pathogenesis in non-clinical models

Trent Ross[1], Kenneth Kelly[1], David Beebe[1], Collin Crowley[1], Morris Birnbaum[1], Jeffrey Pfefferkorn[1], William Esler[*1]

[1]Internal Medicine Research Unit, Pfizer, Cambridge. MA, United States

Corresponding author's email: william.esler@pfizer.com

Introduction: Steatosis results from an imbalance in lipid production/uptake into the liver and clearance/removal. Acetyl-CoA carboxylase (ACC) catalyzes the first committed step in de novo lipogenesis (DNL) and also regulates fatty acid oxidation (FAOX). Pharmacologic inhibition of ACC activity in the liver is hypothesized to reduce steatosis by suppression of lipid production, through inhibition of DNL, and promotion of lipid clearance, through stimulation of hepatic FAOX. Emerging data also suggest that suppression of DNL through ACC inhibition may directly reduce inflammation by selectively restraining the formation of inflammatory Th17 cells, but not anti-inflammatory Treg cells.

Aims: To evaluate the effect of pharmacologic ACC inhibition on steatosis, hepatic inflammation and fibrogenesis in human derived and rodent model systems.

Material and Methods: PF-05221304 (PF) is a potent hepatoselective ACC1/2 inhibitor. The effect of PF on lipid metabolism and lipid accumulation was assessed in primary human hepatocytes and in two rat dietary steatosis models. In addition, the direct effect of PF on inflammation was assessed by measuring polarization of primary human T-cells to inflammatory and anit-inflammatory T-cells in vitro and markers of hepatic inflammation and fibrogenesis in the DEN rat hepatic injury model in vivo.

Results: PF potently inhibited rat and human ACC1/2 in vitro. In isotopic labeling studies, PF inhibited DNL and stimulated FAOX in primary human hepatocytes, resulting in reduced TG accumulation. In acute rat in vivo studies, oral administration of PF selectively suppressed production of malonyl-CoA, the product of ACC, and DNL in liver relative to other tissues in a dose and concentration-dependent manner. In chronic (4 to 6 week) in vivo studies, PF inhibited hepatic DNL and ameliorated steatosis in Western diet fed rats and high-fat fed rats in a dose-dependent manner. In primary human IL-17 secreting T-cells, PF inhibited DNL and restrained polarization to inflammatory Th17 cells but not to anti-inflammatory FoxP3 (+)Treg cells. Administration of PF in the rat DEN liver injury model reduced IHC staining for CD3, a T-cell maker, and aSMA, a myofibroblast activation marker. Total circulating and CD43++ monocytes, ALT levels and liver weights were also reduced with PF administration showing improvements in hepatic necro inflammation.

Conclusions: Hepatic ACC inhibition may reduce steatosis, inflammation and fibrogenesis and have utility for the treatment of NASH.

Disclosure of Interest: T. Ross: Employee: Pfizer, K. Kelly: Employee: Pfizer, D. Beebe: Employee: Pfizer, C. Crowley: Employee: Pfizer, M. Birnbaum: Employee: Pfizer, J. Pfefferkorn: Employee: Pfizer, W. Esler: Employee: Pfizer