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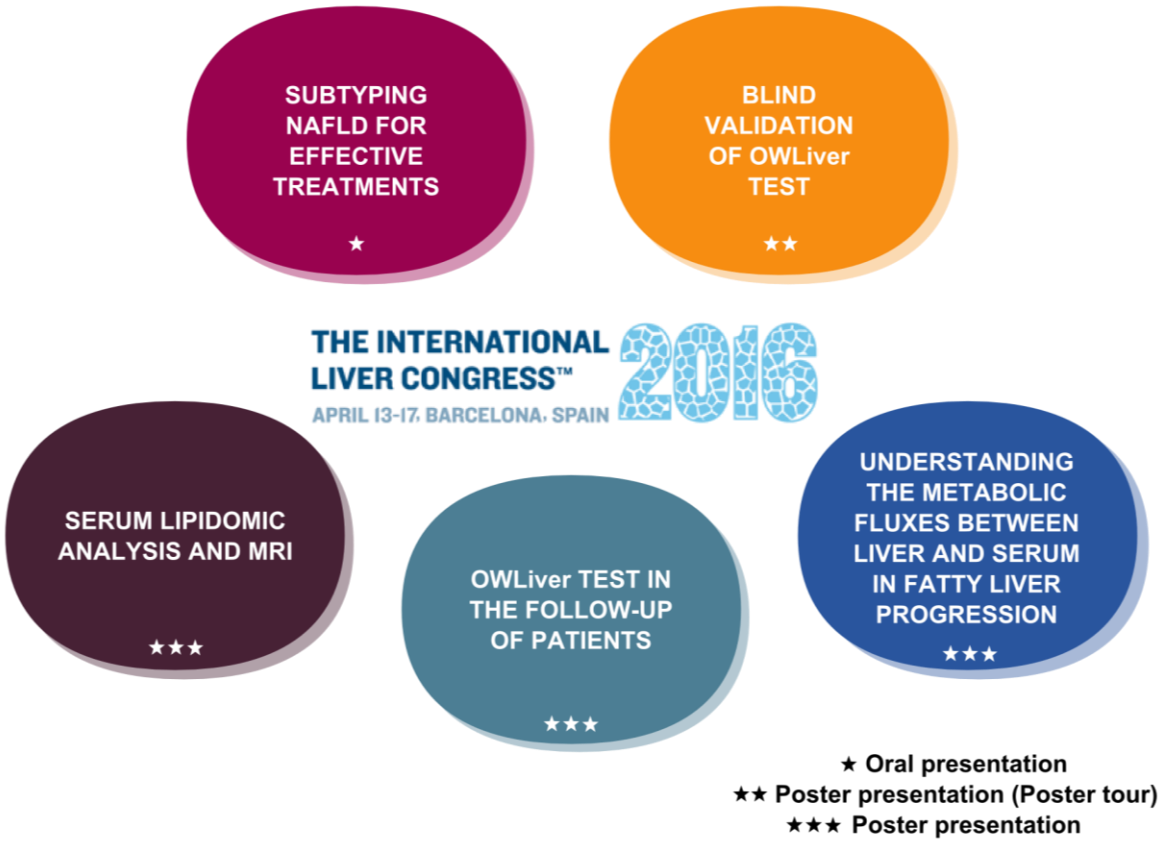

IDENTIFICATION OF LIPIDOMIC SIGNATURES THAT DEFINE THREE SPECIFIC SUBTYPES OF NAFLD AND DIFFERENTIATE NASH FROM SIMPLE STEATOSIS

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Background and Aims: Nonalcoholic steatohepatitis (NASH) is a histological definition that groups together defects in diverse biochemical processes causing hepatic fat accumulation, inflammation, necrosis and fibrosis. The identification of the types of mechanisms leading to NASH and the discovery of noninvasive biomarkers of NASH subtypes are central for the development of effective treatments and precise diagnosis. This study aims to capture the metabolic architecture of the main NASH subtypes to help define effective treatments and discover specific serum metabolomic patterns reflective of each NASH subtype.
Methods: We have compared the serum metabolome (over 400 different molecular species) of a mouse model (Mat1a-/-) that spontaneously develops NASH with that of WT mice and selected the top fifty metabolites that more significantly differentiated between both genotypes (p<1E-05).
Results: Silhouette cluster analysis revealed that this metabolic signature sub-classified a cohort of 377 patients with biopsy proven NAFLD (246 diagnosed of steatosis and 131 diagnosed of NASH) into three clusters: a first cluster (n=116) showing a serum metabolic profile similar to that observed in the Mat1a-/- mice (M-subtype), a second cluster (n=115) showing the opposite metabolomic profile (non-M-subtype) and a third cluster (n=146) presenting an intermediate metabolic profile (I-subtype). Next, we wondered whether NAFLD patients in the M-type NAFLD subgroup could be further separated into simple steatosis and NASH based exclusively in their metabolic profile. Volcano plot analysis [representation of log10(p-value) and log2(fold-change)] identified a group of highly significant lipids (p<1E-05, mostly lysophospholipids and polyunsaturated fatty acids) that effectively differentiated between these two conditions. Similarly, we successfully identified a metabolic signature (mostly triglycerides and phosphatidylcholines) that accurately differentiated between steatosis and NASH in the non-M-type NAFLD subgroup. When this unsupervised approach was applied to the third cluster (I-subtype), the metabolic signature able to differentiate between steatosis and NASH was mainly based in polyunsaturated fatty acids.
Conclusions: Our data document the power of serum metabolomics to identify signatures that define NAFLD subtypes and differentiate NASH from simple steatosis in humans. Moreover, these metabolic signatures may be used to analyze the individual response to treatment in clinical trials.

Corresponding author: José M Mato
Presenting author: Cristina Alonso
Session: Fatty Liver Disease: Experimental
Oral presentation: Friday 15 April at 16:30
Location: Hall 8.0-D1

A NON-INVASIVE LIPIDOMIC TEST ACCURATELY DISCRIMINATES NASH FROM STEATOSIS: A BLIND VALIDATION STUDY

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver diseases worldwide and includes a broad spectrum of histological phenotypes, such as steatosis and steatohepatitis (NASH). While liver biopsy is the reference gold-standard for NAFLD diagnosis, it has the limitations due to its sampling variability, invasive nature and the high cost. Thus, there is a need for non-invasive, robust and reliable, as well as cost-effective procedure. Recently, we have described a serum-based, body mass index-dependent lipidomic signature associated to NAFLD, including 467 biopsy proven NAFLD patients. The aim of this study was to validate this non-invasive test in NAFLD diagnosis using blind-histology as a reference standard.

Methods: Five hospitals were involved in the enrolment of a new blind, biopsy-proven NAFLD cohort to validate the lipidomic test. Finally, 181 patients were included (healthy liver=30; steatosis=66; NASH=85). The serum lipidomic profile was analysed by UPLC-MS. The developed lipidomic test for NAFLD diagnosis was based in two logistic regression algorithms, firstly discriminating between healthy liver and NAFLD (named OWLiver Care), and secondly between NASH and simple steatosis (named OWLiver). The diagnostic performances of both tests were assessed by area under the receiver operating characteristic curve (ROC), sensitivity (se), specificity (sp), positive and negative predictive value (PPV and NPV): 0.90±0.02, 0.98, 0.78, 0.89 and 0.88, respectively for the first discrimination between healthy liver and NAFLD; 0.95±0.01, 0.83, 0.94, 0.89 and 0.90 for the discrimination between NASH and simple steatosis.

Results: Overall, the enrolled subjects were 39% female, aged 46 years and obese (mean BMI=34). Applied to this independent cohort, the performance of the discrimination between healthy liver and NAFLD remained similar, improving the specificity and PPV: AUC=0.91±0.04, se=0.92, sp=0.87, PPV=0.97 and NPV=0.68. The performance of the discrimination between NASH and steatosis decreased slightly, as expected in an independent validation cohort, although the specificity improved as in the previous step of the test: AUC=0.88±0.03, se=0.78, sp=0.95, PPV=0.95 and NPV=0.76.

Conclusions: The results obtained in the independent validation cohort supports that the assessed non-invasive lipidomic tests accurately discriminates firstly between healthy liver and NAFLD, and secondly between NASH and simple steatosis.

Corresponding author: Radan Bruha
Presenting author: Cristina Alonso
Poster: FRI-289
Poster Session: Genetic and metabolic liver disease (Booth 4)
Presentation scheduled in the poster tour entitled: "Fatty liver disease: Clinical" on Friday April 15th from 12:30 - 13:00 guided by Andreas Geier
Porter displayed Friday April 15th between 08:00 and 18:00

NOVEL SERUM LIPIDOMIC SIGNATURE CORRELATES WITH THE GRADE OF STEATOSIS MEASURED BIOCHEMICALLY AND BY MAGNETIC RESONANCE IMAGING

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide. The semiquantitative estimation of steatosis in liver biopsy is the gold-standard for NAFLD diagnosis; however, this expensive and invasive procedure has limitations such as semiquantitative nature and interindividual estimation variability. Finally, it allows quantification of steatosis only in a small sample of the liver, not in the whole organ. Recently [Jimenez-Agüero R et al. BMC Medicine 2014], we developed a novel MRI-based method of quantifying hepatic steatosis which substantially correlates with the hepatic triglyceride concentration (Folch value). Here, we explored potential correlations between serum lipidomic profiling, MRI and Folch value in order to improve NAFLD diagnosis using non-invasive methods.
Methods: In total, 118 obese patients (BMI>35) were included in the study. The grade of steatosis was estimated by histopathology and the biochemical measurement of the hepatic triglyceride concentration (mg of triglyceride/g of liver tissue) was determined according to the Folch method. The liver fat content was also determined by multi-echo MRI. Lipidomic profiling of the serum samples, which were extracted the date of the biopsy and the MRI, was performed by ultraperformance liquid chromatography coupled to mass spectrometry (UPLC-MS). Pearson correlation coefficients were calculated between the lipid species and the Folch value, MRI fat fraction and the grade of steatosis.
Results: Lipidomic profiling in serum revealed 45 species that correlated significantly with the Folch value, MRI fat fraction, and the histopathological examination ($p<0.05$). Among them, we selected a group of 16 lipids with a strong correlation with the Folch value, as well as with the MRI fat fraction (both $p<0.001$). The selected metabolites included seven triglycerides with the carbon number between 48 and 54, and saturated or with a low degree of unsaturations ($p<0.00001$ for the correlation with MRI fat fraction). Additionally, seven glycerophospholipids (phosphocholines and phosphoinositols) and two sphingomyelins correlated ($p<0.02$) with the grade of steatosis.
Conclusions: Lipidomic profiling of serum samples showed a strong correlation with the grade of steatosis measured biochemically and by MRI in obese patients. We selected a potential lipidomic signature of 16 lipid species able to predict the Folch value, MRI fat fraction and histopathological steatosis in obese patients with NAFLD.

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Presenting author: Jesús M. Bañales
Poster: FRI-304
Poster Session: Genetic and metabolic liver disease (Booth 4)
Friday 15 April, 12:30-14:00 (displayed between 08:00 and 18:00)

CLINICAL VALIDATION OF A NON-INVASIVE LIPIDOMIC TEST FOR THE DIAGNOSIS OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in most developed countries. NAFLD ranges from simple steatosis to the appearance of inflammation, cell damage and progressive fibrosis in the more severe non-alcoholic steatohepatitis (NASH) condition, which may also evolve to cirrhosis and hepatocellular carcinoma. The prevalence of NAFLD is high and its incidence has recently risen in most countries due to the current obesity epidemic. There is a clear need for non-invasive markers that allow the diagnosis of this disease without the practice of a liver biopsy. The objective of this study is to assess, firstly, the ability of the OWLiver diagnostic test to discriminate between simple steatosis and steatohepatitis (diagnostic value) and, secondly, its predictive value in the monitoring and prognosis of NAFLD (prognostic value).
Methods: A total of 22 clinicians from 6 hospitals and 1 primary care centre from the Basque Country Public Health System participated in this study, in which 98 NAFLD patients were recruited. Patients were prescribed diet and exercise and were monitored for 18 months, with visits at 0, 9 and 18 months, when serum was collected and metabolic profiling performed. Preliminary results for 77 patients that already finished follow-up are presented here.
Results: 40 females and 37 males with a mean age of 50 years (sd: 10.0) and a mean body mass index (BMI) of 30.2 kg/m2 (sd: 11.3) were studied. OWLiver results were consistent with those obtained from biochemical and anthropometric analyses. They were also consistent with the adherence of some of these patients (19%) to diet and exercise. Changes in the metabolic profile of some patients were obvious and shifts from both NASH to steatosis or steatosis to a healthy liver were observed. Only in patients with high fibrosis or cirrhosis the test did not perform well, due to the resemblance of the metabolic profile of these patients to those with a normal liver.
Conclusions: The OWLiver test is able to non-invasively discriminate between steatosis and NASH, using just a serum sample and avoiding a liver biopsy. The test is less accurate in extreme cases, such as patients with a high amount of fibrosis or cirrhosis. The test emerges as a highly useful tool for specialists to identify those patients with steatosis or normal liver and direct them to primary care, allowing the specialist to focus on patients either with NASH or at risk of developing this more severe condition.

Corresponding author: Javier Bustamante
Presenting author: Patricia Salvador
Poster: FRI-323
Poster Session: Genetic and metabolic liver disease (Booth 4)
Friday 15 April, 12:30-14:00 (displayed between 08:00 and 18:00)

LIPIDOMIC CONNECTIONS BETWEEN LIVER AND SERUM DURING THE ONSET OF NAFLD IN HUMANS

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Background and Aims: Human lipidome is being extensively studied to prevent NAFLD. However, the metabolic fluxes of lipids between liver and serum remain poorly understood in the different stages of lipid accumulation, especially in the onset of fatty liver progression. These metabolite fluctuations must be identified in order to anticipate fatty liver pathophysiological events. Liver lipidome could be deciphered by means of serum lipid profiling, hence, avoiding the inconveniences caused by an invasive biopsy. Afterwards, the lipidomic fluxes between liver and serum during the initial stages of NAFLD have been pinpointed.
Methods: 77 biopsied morbid obese patients were classified as having histological steatosis and their clinical and biochemical features were assessed. Methanol and chloroform/methanol serum and liver extracts were analysed by reverse ultra-high performance liquid chromatography coupled to time-of-flight mass spectrometry (UHPLC-ToF-MS). Studied lipids were classified as fatty *acyls*, bile acids, steroids, lysoglycerophospholipids, glycerolipids, glycerophospholipids, sterol lipids and sphingolipids. Approximately, 400 metabolites were tracked in the serum and liver of these patients and their concentration trends and the metabolite product-to-substrate ratios were compared per patient. Univariate, multivariate, and correlation analyses were also applied.
Results: The concentration of 15 metabolites was strongly correlated between liver and serum in the studied morbid obese patients with liver steatosis. Among them, the cholesteryl ester 20:5, 8 triacylglycerols and 6 diacylglycerophosphocholines with long, saturated and monounsaturated fatty acyl chains, had a positive correlation between 0.7 and 0.82 with significance below 10-9. Captivatingly, the concentration of triacylglycerols with longer acyl chains and more unsaturations presented a marked, gradual increase in the liver and serum of the patients. In addition, the ratio of phosphatidylcholines with docosahexaenoic acid to total phosphatidylcholines increased in parallel between serum and liver of these cohorts. This finding confirms that the activity of phosphatidylethanolamine N-methyltransferase is robustly involved in the setting up of NAFLD.
Conclusions: The specific cholesteryl ester, triacylglycerols and diacylglycerophosphocholines that correlate between liver and serum in morbid obese patients with steatosis represent the serum lipidomic fingerprint of the onset of NAFLD.

Corresponding author: Antonio-Martín Duce
Presenting author: Ainara Cano
Poster: FRI-273
Poster Session: Genetic and metabolic liver disease (Booth 4)
Friday 15 April, 12:30-14:00 (displayed between 08:00 and 18:00)