OWL Abstracts

at

OWL will be presenting new innovations in liver disease diagnosis at The Liver Meeting® in Boston.

Oral presentation: Metabolomics in a liquid biopsy provides a noninvasive comprehensive NAFLD-diagnostic tool

Corresponding author: Arun J. Sanyal
Presenting author: Puneet Puri
Parallel D (Session 5): NAFLD: Diagnosis and Natural History
Moderators: Silvia C. Sookoian, MD, PhD and Rohit Loomba, MD
Sunday, November 13, 1:15 PM-2:45 PM

Poster presentation: A non-invasive lipidomic test accurately discriminates NASH from steatosis and tracks evolution of the disease

Corresponding author: Rocío Aller
Presenting author: Miriam Pérez-Cormenzana
Poster: 1108
Session: Steatohepatitis: Clinical and Therapeutic
Porter displayed November 12, 2016 from 2:00 PM to 7:30 PM
Presenters Available: 5:30 PM–7:00 PM
Room: Poster Hall – Hall C

Poster presentation: Metabolomic classification of murine and human nonalcoholic fatty liver disease

Corresponding author: José M. Mato
Presenting author: Cristina Alonso
Poster: 1603
Session: Steatohepatitis: Experimental II
Porter displayed November 13, 2016 from 8:00 AM to 5:30 PM
Presenters Available: 12:30 PM–2:00 PM
Room: Poster Hall – Hall C

METABOLOMICS IN A LIQUID BIOPSY PROVIDES A NONINVASIVE COMPREHENSIVE NAFLD-DIAGNOSTIC TOOL

Cristina Alonso[1], Puneet Puri[2], Ibon Martinez-Arranz[1], Jesús Bañales[3], Michael O. Idowu[4], Azucena Castro[1], Collaborative Group NASH metabolomics[5], Mazen Noureddin[6], Pierre Bedossa [7], José M. Mato[8], Arun J. Sanyal[2]

[1] OWL, Derio, Spain; [2] Division of Gastroenterology and Hepatology, Virginia Commonwealth University Medical Center, Richmond, VA, United States; [3] Department of Liver and Gastrointestinal Diseases, Biodonostia Research Institute, Biodonostia Research Institute, Donostia University Hospital, University of the Basque Country (UPV-EHU), CIBERehd, IKERBASQUE, Donostia, Spain; [4] Division of Surgical Pathology, Department of Pathology, Virginia Commonwealth University School of Medicine, Richmond, VA, United States; [5] Collaborative Group for NASH metabolomics, J.-F. Dufour, M. Romero Gómez, J. Caballeria, R. Bruha, J. Crespo, R. Aller, J. Muntané, C. García-Monzón, A. Martín-Duce, O. Lo Iacono, R. J. Andrade, M. Pérez-Carreras, K. Clement, P. Gual, Raul Jiménez-Agüero, Luis Bujanda, Derio, Spain; [6] Division of Gastroenterology, Comprenhensive Transplat Center, Cedars-Sinai Medical Center, Los Angeles, CA, United States; [7] Department of Pathology, Hôpital Beaujon, Université Paris Diderot, Paris, France; [8] CIC bioGUNE, CIBERehd, Derio, Spain.

BACKGROUND: Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease. Subjects with nonalcoholic steatohepatitis (NASH) and fibrosis are generally targeted for therapeutic intervention. There is thus an urgent need for point-of-care diagnostics to identify such individuals.
HYPOTHESIS: The metabolic changes associated with development of nonalcoholic fatty liver (NAFL) and NASH are reflected in the circulating metabolome which can be leveraged for diagnostics development.
AIMS: To develop a model based on serum and/or plasma metabolomic analyses to: (1) differentiate NAFLD from controls, (2) assess severity of steatosis, (3) distinguish between NAFL *vs.* NASH, and (4) identify the presence of any fibrosis or advanced fibrosis (stage 3 or 4) in NAFLD.
METHODS: An initial cohort (n=652) was used to develop the models. A validation in independent cohorts (n=295) from the USA and Europe or leave-one-out cross validation (LOOCV) were performed on the models. In a subset of the test cohort (n=114) concomitant hepatic fat content using MR-fat fractions was available to relate the model to hepatic triglyceride content. Serum and EDTA-plasma samples were collected under fasting conditions at the time of liver biopsy. Metabolomic analyses were performed as previously described (J Prot Res 2012,11:2521). Liver histology was assessed by NASH CRN criteria.
RESULTS: A total of 817 NAFLD patients and 130 controls were studied. NAFLD Diagnosis: A BMI-dependent model discriminated between controls and NAFLD (controls=90; NAFLD=377; AUC=0.90±0.02). Applied to the validation cohort, the performance of the model was AUC=0.93±0.03 (Table). Steatosis Severity: There was a strong concordance with hepatic triglyceride content (r=0.81, p<0.0001). NASH diagnosis: NAFL (n=246) and NASH (n=131) could be distinguished by a BMI-dependent model (AUC=0.95±0.01). The AUC was 0.84±0.03 in the validation cohort (Table). Fibrosis Assessment: It was performed in 185 NAFLD patients (F0=71; F1&F2=80; F3&F4=34). Two algorithms were found that discriminated between F0 *vs.* F1-4 (AUC=0.92±0.02; LOOCV: AUC=0.85) and between early and advanced fibrosis (AUC=0.89±0.03; LOOCV: AUC=0.86).
CONCLUSIONS: These data provide proof of concept that liquid biopsy metabolomics can be used to resolve diagnostic questions in NAFLD management.

	NAFLD *vs.* Control (OWLiver Care)					
	Total	Control	NAFLD	AUC	PPV	NPV
Test (N)	467	90	377	0.90±0.02	0.89	0.88
Validation (N)	295	40	255	0.93±0.03	0.97	0.79
	NAFL *vs.* NASH (OWLiver)					
	Total	NAFL	NASH	AUC	PPV	NPV
Test (N)	377	246	131	0.95±0.01	0.89	0.90
Validation (N)	255	108	147	0.84±0.03	0.93	0.76

A NON-INVASIVE LIPIDOMIC TEST ACCURATELY DISCRIMINATES NASH FROM STEATOSIS AND TRACKS EVOLUTION OF THE DISEASE

Miriam Perez-Cormenzana[1], M Concepción García[3], Beatriz Antolin[3], Natalia Mora[3], Ibon Martinez-Arranz[1], De luis D. Román[2], Cristina Alonso[1], Rocío Aller[3,2]

[1] OWL, Derio, Spain; [2] Institute of Endocrinology and Nutrition, Medicine School and Department of Endocrinology and Nutrition, Hospital Clínico Universitario, University of Valladolid, Valladolid, Spain; [3] Department of Digestive Disease, Hospital Clínico Universitario, University of Valladolid, Valladolid, Spain.

BACKGROUND: Nonalcoholic fatty liver disease (NAFLD) includes a spectrum of histological phenotypes including steatosis, steatohepatitis (NASH) and fibrosis. While liver biopsy is the reference for diagnosis, it is invasive and associated with procedural risks and sampling variability. Thus, there is urgent need for a noninvasive and robust diagnostic procedure. Recently, we have described a serum-based lipidomic signature associated with NAFLD able to fulfill these unmet clinical diagnostic needs by: (1) differentiating NAFLD from healthy cohort, (2) discriminating between steatosis and NASH.
AIMS: To validate this non-invasive assay in NAFLD diagnosis using blind-histology as a reference standard and then apply these test in the follow-up of the patients.
METHODS: Thirty patients were enrolled as a blind, biopsy-proven NAFLD cohort, collecting the serum samples at the time of liver biopsy. Metabolic syndrome was assessed based on the presence of at least three of the conditions listed by the NCEP ATPIII. Patients were prescribed a hypocaloric diet (1500kcal/day) and aerobic exercise (30-60min/day), monitored for 2 to 5 years, at which point a new serum sample was collected. The lipidomic test was established on the basis of 467 biopsy-proven patients (controls=90; steatosis=246; NASH=131) and two BMI-dependent logistic regression algorithms: 1) discriminating between NAFLD and healthy liver (assay name: OWLiver Care) and 2) between NASH and steatosis (OWLiver). The diagnostic performances of both assays were assessed by area under the ROC curve, positive and negative predictive values: 1) 0.90±0.02, 0.89 and 0.88, respectively; 2) 0.95±0.01, 0.89 and 0.90.
RESULTS: Applied to the independent biopsy-proven cohort (46±12years, 33%female, weigh=86±15kg; BMI=32±5kg/m2), the test diagnosed correctly 28 out of 30 patients, misclassifying one patient having NASH with NAS score=2, but presenting metabolic syndrome; and one patient as having steatosis with NAS score=5, although without ballooning. Once validated, the test was applied to the follow-up of the patients (weigh=84±14kg; BMI=31±4kg/m2). 31% of the patients lose at least 5% of baseline body weight. Among those responders, 50% of them improved their diagnosis presenting positive post-interventional shifts from NASH to steatosis or steatosis to healthy liver. Interestingly, the original diagnosis remained unchanged for the 95% of the non-responder patients.
CONCLUSIONS: The results obtained in the independent cohort support the feasibility of these lipidomic tests as a noninvasive tool for NAFLD diagnosis and to monitor the disease progression/regression while circumventing the need for repeat liver biopsy.

METABOLOMIC CLASSIFICATION OF MURINE AND HUMAN NONALCOHOLIC FATTY LIVER DISEASE

Cristina Alonso[1], Jose L. Lavin[2], Marta Iruarrizaga-Lejarreta[1], David Fernández[3], Sebastiaan van Liempd[2], Laura delaCruz-Villar[2], Nicolás Navasa[2], Ibon Martinez-Arranz[1], Igor Aurrecoechea[4], Xavier Buqué[4], Marta Varela[3], Juan M. Falcon-Perez[5], Elizabeth P. Newberry[6], Patricia Aspichueta[4], Ana M. Aransay[3], Mazen Noureddin[7], Juan Anguita[8], Nicholas O. Davidson[6], Maria L. Martinez-Chantar[3], Shelly C. Lu[7], José M. Mato[3]

[1] OWL, Derio, Spain; [2] CIC bioGUNE, Derio, Spain; [3] CIC bioGUNE, CIBERehd, Derio, Spain; [4] Faculty of Medicine and Nursery, University of the Basque Country, Leioa, Spain; [5] CIC bioGUNE, CIBERehd, Ikerbasque, Derio, Spain; [6] Department of Medicine and Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO, United States; [7] Division of Gastroenterology and Hepatology, Cedars-Sinai Medical Center, Los Angeles, CA, United States; [8] CIC bioGUNE, Ikerbasque, Derio, Spain.

Purpose of Study: Increasing evidence points to different subtypes of nonalcoholic fatty liver disease (NAFLD) which progress to nonalcoholic steatohepatitis (NASH) and fibrosis at different rates and may respond differently to treatment. We asked if different pathways causing NASH yield distinct serum metabolomic signatures. **Methods:** We undertook lipidomic (over 500 metabolites) and transcriptomic (RNA Seq) analysis in three mouse models of NASH and fibrosis: 1) High fructose/high trans fat (HFTF) fed C57BL6/J (WT); 2) Germline methionine adenosyltransferase 1a knockout (Mat1a KO); and 3) WT mice fed a methionine, choline deficient (MCD) diet. **Results:** All groups showed increased liver fatty acid (FA) uptake and accumulation of FA, triglyceride and cholesterol ester, and histological NASH and fibrosis versus appropriate controls. However, the groups differed substantially in de novo lipogenesis (DNL), FA esterification, mitochondrial, peroxisomal and endoplasmic reticulum (ER) FA oxidation, phospholipid metabolism, phosphatidylcholine (PC)/phosphatidylethanolamine (PE) ratio and VLDL export. Specifically, HFTF mice showed increased DNL, impaired mitochondrial FA-oxidation, increased peroxisomal FA-oxidation, normal PC/PE ratio and VLDL export. By contrast, Mat1a KO mice exhibited normal DNL, reduced mitochondrial FA-oxidation, increased peroxisomal and ER FA-oxidation, reduced PC/PE ratio and VLDL export. Mice fed a MCD diet showed decreased DNL, increased mitochondrial and ER FA-oxidation, reduced PC/PE ratio and impaired VLDL export. Murine serum lipidomic signatures were used as comparators to serum metabolomics signatures of 377 patients with biopsy proven NAFLD (246 steatosis and 151 NASH), the findings showing two distinct subtypes. Subtype I (222 patients) was enriched for MCD and Mat1a KO signatures, while the HFTF signature was distributed across both subtypes. Subtype I was characterized by a reduction in specific PC species, PC(20:0/18:2), PC(18:2/20:4), PC(18:0/22:5), PC(16:0/22:6), PC(18:0/0:0), PC(20:0/0:0), PC(20:1/0:0), while subtype II showed elevations in each of these species. We further identified serum biomarkers that distinguished NASH from steatosis. For example, increased content of plasmalogens PC(P-20:1/0:0), PC(P-18:1/0:0), PC(P-20:2/0:0), PC(P-16:0/0:0) differentiated between NASH and steatosis in NAFLD patients with subtype I, while a decrease in TG(54:6), TG(54:5), PC(17:1/18:1), PC(19:1/18:2) differentiated NASH and steatosis in subtype II. **Conclusions:** These results indicate that the traditional, mainly pathology-driven classification of NAFLD/NASH, can be refined and perhaps represented by metabolomics classification.