A precision medicine approach to comprehensive NAFLD diagnosis via metabolomics-based liquid biopsy

International Workshop on NASH Biomarkers
May 5-6 2017, Washington D.C.
Pablo Ortiz, MD, PhD (OWL CEO)
Clinical Database with histologically-verified Liver biopsy:

- A total of 817 subjects with NAFLD and 130 controls were included
- Cohort of subjects from the USA and Europe
- Liver histology was assessed by NASH CRN criteria
- Serum and EDTA-plasma (n=947) collected under fasting conditions at the time of liver biopsy:
  - Development of the models: An initial cohort of 652 samples
  - Validation: independent cohorts (n=295) or leave-one-out cross validation (LOOCV)
  - In a subset of the serum cohort (n=114) concomitant hepatic fat content using MRI-fat fractions is also available
- Samples have been characterized through comprehensive metabolomics analysis (J Prot Res 2012, 11:2521)
Clinical Validation

Patient cohort – Discovery Trial

- **467 biopsied patients**

- **11 participating hospitals:**
  - Hospital Clinic Barcelona
  - Hospital Marqués de Valdecilla
  - Hospital Virgen de Valme
  - Hospital Universitario Reina Sofia
  - Hospital Universitario Virgen de la Victoria
  - Hospital Universitario 12 de Octubre
  - Hospital Universitario Principe de Asturias
  - Hospital Universitario del Tajo
  - Hospital Universitario Santa Cristina
  - Keck Hospital of USC
  - Institute Nacionale de la Santé et dela Recherche Médicale (INSERM)

ROC Curves – OWLiver 3.0

AUC 0.90 ± 0.02
Sensitivity: 0.98
Specificity: 0.78
Pos Pred Value: 0.89
Neg Pred Value: 0.88

AUC 0.95 ± 0.01
Sensitivity: 0.83
Specificity: 0.94
Pos Pred Value: 0.89
Neg Pred Value: 0.90
Clinical Blind Validation

Patient cohort – Validation Trial

- **294 additional** biopsied patients (new cohort)
- 2 countries: Czech Republic and Spain
- 6 Hospitals
  - Hospital Marques de Valdecilla
  - Hospital Virgen de Valme
  - Hospital Clínico Universitario Valladolid
  - Hospital Clinic Barcelona
  - Donostia University Hospital
  - Faculty of Medicine, Charles Univ. of Prague
- Blind Validation

A Non-Invasive Lipidomic Test Accurately Discriminates Non-Alcoholic Steatohepatitis from Steatosis: A Blind Validation Study
Journal of Hepatology, Vol. 64, Issue 2, S478
Serum metabolic biomarkers of NAFLD

Study group: 467 biopsied subjects with
Normal Liver (n=90) or NAFLD (377): Steatosis (246), NASH (131)

NAFLD index = \( f(\text{BMI, relative concentration of 11 TG}) \)
AUROC = 0.90; Sensitivity = 0.98; Specificity = 0.78; PPV = 0.89; NPV = 0.88

Validation group: 294 biopsied subjects with
Normal Liver (n=39) or NAFLD (n=255): Steatosis (108), NASH (147)

NAFLD index = \( f(\text{BMI, relative concentration of 11 TG}) \)
AUROC = 0.95; Sensitivity = 0.96; Specificity = 0.82; PPV = 0.97; NPV = 0.78

Journal of Hepatology, Vol. 64, Issue 2, S478
NASH diagnosis: Validation

Study group: 540 metabolites were profiled by LC/MS in serum samples from 377 biopsied NAFLD subjects: Steatosis (246), NASH (131)

NASH index = \int (\text{BMI}, \text{relative concentration of 20 TG})
AUROC = 0.95; Sensitivity = 0.83; Specificity = 0.94

Validation group: 255 biopsied NAFLD subjects: Steatosis (n=108), NASH (147)

NASH index = \int (\text{BMI}, \text{relative concentration of 20 TG})
AUROC = 0.84; Sensitivity = 0.79; Specificity = 0.92
Noninvasive diagnosis of NAFLD and NASH based on lipidomics

Discovery and validation of test based on twenty-eight serum triglycerides

**NAFLD vs. Normal Liver**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Control</th>
<th>NAFLD</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test (N)</td>
<td>467</td>
<td>90</td>
<td>377</td>
<td>0.90±0.02</td>
</tr>
<tr>
<td>Validation (N)</td>
<td>118</td>
<td>16</td>
<td>102</td>
<td>0.87±0.04</td>
</tr>
</tbody>
</table>

**NASH vs. NAFL**

<table>
<thead>
<tr>
<th></th>
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<th>NASH</th>
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<td>Test (N)</td>
<td>377</td>
<td>246</td>
<td>131</td>
</tr>
<tr>
<td>Validation (N)</td>
<td>95</td>
<td>43</td>
<td>52</td>
</tr>
</tbody>
</table>
Fibrosis Assessment

Non-invasive assay for tracking severity of the disease based on plasma metabolomics

**Discovery Trial**
Plasma samples from USA:
VCU (Dr. Sanyal)
**Patients: 105**

**Validation Trial**
Samples from Switzerland and Spain:
Inselspital - Bern University Hospital (Dr. Dufour)
H. Universitario de Valme (Dr. Romero)
H. Universitario Santa Cristina (Dr. García-Monzón)
H. Universitario Príncipe de Asturias (Dr. Martín-Duce)
**Patients: 120**

Central reading of all 225 patients at VCU.
Final results are based in a total of 208 NAFLD patients.
Fibrosis Assessment: NAFLD progression

Heatmap combining discovery and validation cohorts
Noninvasive fibrosis assessment

<table>
<thead>
<tr>
<th>Total (N)</th>
<th>N</th>
<th>AUC</th>
<th>AUC (LOOCV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0 vs. F1-4</td>
<td>185</td>
<td>71 (F0)</td>
<td>114 (F1-4)</td>
</tr>
<tr>
<td>F0-1 vs. F2-4</td>
<td>185</td>
<td>140 (F0-1)</td>
<td>45 (F2-4)</td>
</tr>
<tr>
<td>F1 vs. F2-4</td>
<td>114</td>
<td>69 (F1)</td>
<td>45 (F2-4)</td>
</tr>
<tr>
<td>F1-2 vs. F3-4</td>
<td>114</td>
<td>80 (F1-2)</td>
<td>34 (F3-4)</td>
</tr>
</tbody>
</table>

16 metabolites were included: phospholipids, triglycerides and non-esterified fatty acids
Steatosis severity

- 114 biopsied patients
- Obese population

Correlation between Metabolic Serum Profile and MRI

Correlation between the Lipidomic Signature and MRI ($r=0.81$, $p<0.0001$)
Aims: A Comprehensive NAFLD Diagnosis

To develop metabolomics based models on liquid biopsy to

1. Differentiate NAFLD from controls
   **NAFLD Diagnosis (Screening)**

2. Assess severity of **Steatosis**

3. Distinguish between NAFL and NASH
   **NASH Diagnosis**

4. Identify the presence of **Any Fibrosis** or **Advanced Fibrosis** (stage 3 or 4) in NAFLD
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