Translational strategies for the study of chronic liver injury

FIF Scientific Committee Annual Meeting - 19/11/2015

Natalia Rosso
NAFLD/ALD
- Steatosis
- Lipotoxicity
- Inflammation
- Oxidative stress

NASH/ASH
- Hepatocyte injury
- HSC activation
- Fibrogenesis

Cirrhosis
- Liver fibrosis
- Functional complications

HCC
- End stage liver damage

**Therapy**

- Therapeutic approach for NASH: silimar properties
  
  *Veronica Marin, Sabrina Gambaro, Silvia Gazzin*

**Pathophysiology**

- Role of chronic Inflammation in the progression of NAFLD – (WAT-Liver axis)
  
  *Carla Chackelevicius*

- MIF in the progression of ALD – cross talk between macrophages and hepatocytes
  
  *Veronica Marin*

- Gut–liver axis: role of microbiota
  
  *Veronica Marin, Silvia Gazzin, Manola Comar*

**Diagnosis**

- In silico and in vivo validation of novel noninvasive biomarkers for liver Fibrosis
  
  *Pablo Giraudi, Sabrina Gambaro, Sofia Ornelas Arroyo*
Role of Chronic Inflammation in Non Alcoholic Fatty Liver Disease

Dipartimento Chirurgia Generale
Ospedale di Cattinara
Silvia Palmisano
Michela Giuricin
Nicolò de Manzini

School of Anatomic Pathology,
Department of Medical, Surgical and Health Sciences, University of Trieste
Deborah Bonazza
Fabrizio Zanconati
The lymphocyte Th17/T-regulatory imbalance

An imbalance in Th17/T-regulatory cells is associated with liver fibrosis, by promoting inflammation and Hepatic Stellate Cell activation.

WAT from metabolically abnormal obese presented higher levels of CD4 T cell (Th17 phenotype) than metabolically normal subjects.

The role of Th17 cells in HUMAN liver disease is not fully understood.
Samples from patients undergoing **bariatric surgery**

**Liver**
- Histopathological Score
  - Steatosis; Inflammation; Fibrosis
- Biochemical parameters
  - ALT; AST; GGT; Lipids; Glucose homeostasis
- Molecular analysis (RT-PCR)
  - IL1β; IL8; TNF-α; VEGFA
  - TGF-β; α-SMA; Col1A1;
  - RORC; IL17; IL17RA; POSTN
- Characterization of Inflammatory infiltrates
  - Th17 subpopulation

**White Adipose Tissue**
- Molecular analysis (RT-PCR)
  - IL-17; IL-17RA; RORC; TNF-α
- Characterization of Inflammatory infiltrates
  - Th17 subpopulation

**Whole Blood**
- Cytokines plasmatic quantification
  - IL-17
- Isolation of peripheral cells
  - Identification of Th17 subpopulation
## Cohort characteristics

<table>
<thead>
<tr>
<th>Patients</th>
<th>n</th>
<th>Age</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48</td>
<td>44.5</td>
<td>44.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(19-63)</td>
<td>(34.6-70.4)</td>
</tr>
<tr>
<td>Surgery</td>
<td>23</td>
<td>GBP</td>
<td>25 Sleeve</td>
</tr>
</tbody>
</table>

## Liver Histopathological findings

<table>
<thead>
<tr>
<th>Steatosis</th>
<th>n</th>
<th>Fibrosis</th>
<th>n</th>
<th>Inflammation</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 (63%)</td>
<td>0</td>
<td>2 (4%)</td>
<td>1</td>
<td>20 (42%)</td>
</tr>
<tr>
<td>2</td>
<td>16 (33%)</td>
<td>1</td>
<td>38 (79%)</td>
<td>2</td>
<td>11 (23%)</td>
</tr>
<tr>
<td>3</td>
<td>1 (2%)</td>
<td>2</td>
<td>7 (15%)</td>
<td>On Going</td>
<td>17 (35%)</td>
</tr>
<tr>
<td>On Going</td>
<td>1 (2%)</td>
<td>On Going</td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Preliminary Results – *(FO vs F1)*

**Inflammation markers**
- ↑ VEGFA; ↑ IL1β; ↑ IL8

**Fibrosis markers**
- ↑ α-SMA; ↑ TGFβ; ↑ Col1A1

**Th17 pathway**
- ↑ RORC; ↑ IL-17; ↑ IL17RA

**Inflammatory infiltrates**
- On going

**Plasmatic Cytokines level**
- IL17 unchanged

**Isolation of peripheral cells**

**Inflammation marker**
- ↑ TNFα

**Th17 pathway**
- ↑ RORC; ↑ IL-17; ↑ IL17RA

**Inflammatory infiltrates**
- On going
Therapeutic approach to NAFLD/NASH: is Silymarin able to ameliorate liver injury?
Silymarin

Silymarin is the mixture of flavolignans extracted from fruits and seeds of Milk Thistle. The main active compound in Silybin.

Properties reported in literature¹,²:

✓ Free radical scavenging
✓ Inhibition of lipid peroxidation
✓ Reduction of cholesterol and LDL plasmatic levels
✓ Stimulation of protein synthesis
✓ Hepatoprotection in poisoning by *Amanita Phalloides*
✓ Reduction of transamines
✓ Modulation of glucose uptake

Experimental Setup Phase II

- HFHC diet
- HFHC diet + Silymarin

- T0
- T1 4 weeks
- T2 8 weeks
- T3 12 weeks
- T4 16 weeks
- Final 20 weeks

Therapeutic actions

Silymarin (Milk Thistle extract) + Coconut oil (vehicle) → Pellet of HFHC + Silimarlin

Dosage: 33 mg/kg/day
Experimental groups

1) CTRL diet

2a) HFHC diet for all trial

2b) HFHC diet and Silymarin 33 mg/kg/day

2d) CTRL diet and Silymarin 33 mg/kg/day

2e) Come back to CTRL diet and coconut oil
Summary MALES

Macrosopic parameters
- Body weight
- Hepatomegaly
- Adipose Tissue
  (Epidydimal fat pads)

Blood parameters
- Insulin Resistance
- Dyslipidemia
- ALT

Histological parameters
- Steatosis
- Inflammation
- Fibrosis

Graphs showing data over time for different parameters.
Summary FEMALES

**Macrosopic parameters**
- Body weight
- Hepatomegaly
- Adipose Tissue
  *(Epidydimal fat pads)*

**Blood parameters**
- Insulin Resistance
- Dyslipidemia
- ALT

**Histological parameters**
- Steatosis
- Inflammation
- Fibrosis

---

**Diagram**

- **Macrosopic parameters**
  - Colored bars represent different parameters over time:
    - Blue: Body weight
    - Green: Hepatomegaly
    - Orange: Adipose Tissue

- **Blood parameters**
  - Purple bar: Insulin Resistance
  - Red bar: Dyslipidemia
  - Green bar: ALT

- **Histological parameters**
  - Gray bar: Steatosis
  - Blue bar: Inflammation
  - Purple bar: Fibrosis

---

**Timeline**

- **12 wks**
- **16 wks**
- **20 wks**
- **4 wks**
- **8 wks**
- **4 wks**
Molecular Analysis

- Lipid metabolism
- Inflammation
- Fibrosis
- Oxidative stress
- Apoptosis
Without any change in life style, Silymarin might exert positive effects with an improvement of the disorder.
Publications and presentations


2. A Novel mouse model for the study of pediatric Non-Alcoholic Fatty Liver Disease (NAFLD) Marin, V; Rosso, N; Dal Ben, M; Raseni, A; Degrassi, C; Tiribelli, C; Gazzin, S. Hepatology Volume 62, Issue Supplement S1 P1231A

3. Therapeutic Approach to NAFLD/NASH: is Silimarina able to ameliorate liver injury? Marin, V; Gambaro, SE; Dal Ben, M; Raseni, A; Degrassi C; Tiribelli, C; Gazzin, S; Rosso, N Digestive and Liver Disease Oct-2015 Vol.47 S3

4. Silibinin as Therapeutic approach in an in vitro model of NAFLD. Marin, V; Tiribelli, C; Rosso N. Digestive and Liver Disease Oct-2015 Vol.47 S3

5. Effect of age and gender in the progression of NAFLD towards NASH in a juvenile mice model. Marin, V; Rosso, N; Dal Ben, M; Raseni, A; Degrassi, C; Tiribelli, C; Gazzin, S. N. Digestive and Liver Disease Oct-2015 Vol.47 S3

6. Characterization of an in vivo model of juvenile non-alcoholic fatty liver disease. Marin, V; Rosso, N; Dal Ben, M; Raseni, A; Degrassi, C; Tiribelli, C; Gazzin, S. N P0975- – April, 2015 J. Hepatology S2 Vol. 62 S711-712
Identification and evaluation of novel non-invasive biomarkers for liver fibrosis
Liver biopsy is the **gold standard** for NAFLD diagnosis and staging of fibrosis

BUT, is impossible to perform liver biopsy to each patient suspected NAFLD (ethical issues)

There are still no reliable **non-invasive diagnostic tests**

**State of the art**

Identification and validation of potential serum biomarkers in NAFLD subjects
In Silico studies (identification of potential biomarkers)

Total Nodes: 1008
Total Edges: 1754

Highly connected nodes (proteins) are called hubs
Clinical characteristics of the morbidly obese cohort

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Morbidly obese</th>
<th>(-) Ctrl</th>
<th>(+) Ctrl</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>47</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Females/males, n</td>
<td>29/18</td>
<td>5/7</td>
<td>7/7</td>
</tr>
<tr>
<td>Age, mean</td>
<td>43.15 ± 12.1</td>
<td>32.67 ± 5.4</td>
<td>64.9 ± 10.5</td>
</tr>
<tr>
<td>BMI</td>
<td>44.5 ± 6.8</td>
<td>21.6 ± 2.1</td>
<td>28.7 ± 4.5</td>
</tr>
<tr>
<td>ALT (IU/L), mean (&gt;35, %)</td>
<td>31 (19.1 %)</td>
<td>20 (8.3 %)</td>
<td>37 (50%)</td>
</tr>
<tr>
<td>AST (IU/L), mean (&gt;35, %)</td>
<td>21.9 (8.5 %)</td>
<td>22.3 (0%)</td>
<td>41.2 (50%)</td>
</tr>
<tr>
<td>GGT (IU/L), mean (&gt; 42, %)</td>
<td>24.7 (8.5%)</td>
<td>19 (0%)</td>
<td>124 (100%)</td>
</tr>
<tr>
<td>Cholesterol (mg/dL), mean (&gt;200, %)</td>
<td>197.7 (44%)</td>
<td>normal</td>
<td>191 (36%)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL), mean (&gt;160, %)</td>
<td>131.9 (27.6%)</td>
<td>normal</td>
<td>136 (36%)</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL), mean (&lt;40, %)</td>
<td>46 (23.4%)</td>
<td>normal</td>
<td>51 (28%)</td>
</tr>
<tr>
<td>HbA1c (%) mean, (&gt;6, %)</td>
<td>6.3 (42.5%)</td>
<td>normal</td>
<td>6.8 (43%)</td>
</tr>
<tr>
<td>HOMA-IR, mean (&gt;3, %)</td>
<td>5.6 (63.8%)</td>
<td>normal</td>
<td>3.6 (50%)</td>
</tr>
</tbody>
</table>
# Hepatic Gene expression- Histology Correlation

<table>
<thead>
<tr>
<th>Function</th>
<th>Biomarker</th>
<th>STEATOSIS</th>
<th>FIBROSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Described marker</td>
<td>CK18</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>IBP3</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>LCP1</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>HSC activation</td>
<td>HACTA</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>LGALS1</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>UBE4B</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>LAMB1</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td>ECM comp</td>
<td>MMP2</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>TIMP2</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>SPP1</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td>Our candidates</td>
<td>CD44</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>SPARC</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>IGF2</td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>EGFR</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
Plasmatic concentration were correlated with liver fibrosis according to Brunt’ score. F0 vs F1, F2 *p<0.01.
Functional activity of MMPs vs. liver fibrosis

Blood plasma samples

MMP2 **p<0.01 (+)Ctrls 3/4 vs Score 0
MMP2. ***p<0.001 (+)Ctrls 3/4 vs Score 1
MMP2 **p<0.01 (+)Ctrls 3/4 vs Score 2
Plasmatic levels of the candidate biomarkers during 1 year of follow-up

**CD44**

- **T0** vs **T6M**: *p<0.05
- **T0** vs **T12M**: **p<0.01
- **T6M** vs **Ctrls**: ***p<0.001

**IGF2**

- **T0** vs **T12M**: **p<0.01
- **T0** vs **Ctrls**: ***p<0.001

**EGFR**

- **T0** vs **T6M**: *p<0.05
- **T0** vs **T12M**: **p<0.001
- **T0** vs **Ctrls**: ***p<0.001

![Graphs showing plasmain levels of CD44, IGF2, and EGFR over time after surgery, with statistical differences indicated.](image-url)
Conclusions

- **IGF2 and EGFR:** differentiate liver fibrosis vs. Healthy lean subjects

- **CD44:** would correlates with fibrosis score (we need to increase the number of patients)

- **Plasmatic activity of MMP2** would differentiate liver fibrosis vs. health lean subjects

- **Follow-up:** CD44, IGF2 and EGFR plasmatic levels improve after surgery being similar to health lean subjects levels after 12 months.

Prospectives

- If confirmed in larger cohorts, these data suggest that this panel of soluble biomarkers would predict the early stages of liver fibrosis
1. Identification and evaluation of novel non-invasive biomarkers for Non-Alcoholic Liver Disease. Giraudi, PJ, Gambaro, SE; Chackelevicius CM; Giuricin, M; Crocè, LC; Bonazza, D; Masutti, F; Belgrano, M; De Manzini, N; Tiribelli, C; Palmisano, S; Rosso, N. *Digestive and Liver Disease* Oct-2015 Vol.47 S3


4. The interplay between hepatic stellate cells and hepatocytes in an in vitro model of NASH. Barbero-Becerra, VJ, Giraudi, PJ; Chávez-Tapia, NC; Uribe, M; Tiribelli, C; Rosso, N -. 2015 Jul *Toxicol In Vitro*;29(7):1753-1758. doi: 10.1016/j.tiv.2015.07.010 PMID:26187275

5. Importanza dell’interazione epatociti - cellule stellate durante il processo di fibrogenesi in un modello in vitro di steatoepatite non alcolica. Giraudi, P. J.; Barbero Becerra, V; Marin, V, Chavez-Tapia, NC ; Tiribelli, C; Rosso, N. *Liver Gymnasium June 18, 2015 Padova-Italy*

6. The importance of the interplay between hepatocytes and hepatic stellate cells during fibrogenesis in a NASH in vitro model. Giraudi, PJ; Barbero Becerra, VJ; Marin, V, Chavez-Tapia, NC; Tiribelli, C; Rosso, N. – *April, 2015 J. Hepatology*

7. Interaction between hepatocytes and hepatic stellate cells as a crucial factor during fibrogenesis in a NASH in vitro model. Giraudi, PJ; Barbero Becerra VJ; Marin, V; Chavez-Tapia, NC; Tiribelli, C; Rosso, N. *Hepatol Int* (2015)

Publications and presentations


2. Macrophage Migration Inhibitory Factor is protective in a model of Chronic-Binge Ethanol Feeding in Mice. Poulsen KE; Rosso, N; Marin, V; McMullen MR; Morris, A; Tiribelli, C; Nagy, LE. Hepatology Volume 62, Issue Supplement S1 P871A

3. Cross talk between hepatocytes and macrophages in Alcohol liver disease: which language do they use? Marin, V; Poulsen KE, Nagy, LE; Tiribelli, C; Rosso, N. Hepatology Volume 62, Issue Supplement S1


NAFLD Group

- Veronica Marin
- Pablo Giraudi
- Sabrina Gambaro
- Carla Chackelevicius

Silvia Gazzin
Matteo Dal Ben

Welcome! → Sofia Ornelas Arroyo

Acknowledgements

thank you!
For your attention !!!