Serum markers of collagen formation are associated with the severity of liver fibrosis and Non-Alcoholic Steatohepatitis (NASH) histological features and to impaired renal function (IRF) in a NAFLD cohort

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Disclosures

• Diana J. Leeming, Federica Genovese and Morten A. Karsdal are full-time employees and stock owners of Nordic Bioscience A/S

• Samuel J Daniels and Rune Vestermark are a full-time employees of Nordic Bioscience A/S

• No further disclosures from other co-authors
Non-Alcoholic Fatty Liver Disease (NAFLD)


20-55% have CKD

CKD- Chronic kidney disease

Biomarker unmet needs in NAFLD and CKD

- **NAFLD**
  - Diagnostic
  - Prognostic
    - Identify individuals at risk of progression
    - Discriminate “fast” vs. “slow” progressors
  - Monitoring
    - Efficacy of intervention
    - Follow progression/regression of disease

- **CKD**
  - Prognostic
    - Identify individuals at risk of progression
  - Monitoring
    - Efficacy of intervention
Fibrosis is not just fibrosis!

Healthy

Pathology

Central vein

Hepatocytes

Portal triad

Bridging fibrosis

Pericellular fibrosis
A COLLAGEN IS NOT JUST A COLLAGEN
There are 28 collagens

Karsdal MA et al, Adv Drug Del Rev, 2017
Aim

To explore the association of type III and VI collagen formation biomarkers (PRO-C3 and PRO-C6) to liver histology parameters and to impaired renal function (IRF).
**PRO-C3 - A neo-epitope marker of true type III collagen formation**

- Type III collagen synthesis is upregulated in liver fibrosis
- PRO-C3 is a neo-epitope marker reflecting true type III collagen formation, released by ADAMTS2
- PRO-C3 is diagnostic as well as related to progression of liver fibrosis in patients with chronic liver disease\(^1,2,3,4\)

3. Daniels et al. NASH- TAG 2018
4. Leeming et al. NASH Biomarker meeting 2017
PRO-C6 – A marker of type VI collagen formation

- Type VI collagen synthesis is upregulated in fibrotic kidneys
- The PRO-C6 assay targets the C-telopeptide of type VI collagen
- PRO-C6 is related to progression of chronic kidney disease, all-cause mortality and cardiovascular events disease¹,²,³

PRO-C6 – A marker of type VI collagen formation

PRO-C6 is present in highly fibrotic areas in the kidneys

Urinary endotrophin predicts disease progression in patients with chronic kidney disease

Patients had a baseline liver biopsy and were scored according to the NASH CRN scoring system.

Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI formula for a sub-population of 121 patients.

PRO-C3 and PRO-C6 were assessed in serum samples using a competitive enzyme-linked immunosorbent assay (ELISA, Nordic Bioscience A/S, Denmark), employing the protein fingerprint technology.

## Patient Demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Median</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>235</td>
<td>53</td>
<td>50.0 to 55.0</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>236</td>
<td>50.5</td>
<td>45.0 to 56.0</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>220</td>
<td>35.0</td>
<td>32.0 to 40.0</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>232</td>
<td>34.0</td>
<td>32.0 to 36.0</td>
</tr>
<tr>
<td>Diabetic</td>
<td>239</td>
<td>36%</td>
<td></td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
<td>197</td>
<td>5.6</td>
<td>5.5 to 5.8</td>
</tr>
<tr>
<td>Fibrosis Stage (0/1/2/3/4)</td>
<td>82/81/29/31/16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>235</td>
<td>60%</td>
<td>Female</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>235</td>
<td>57.0</td>
<td>49.0 to 66.0</td>
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<tr>
<td>HDL (mmol/L)</td>
<td>175</td>
<td>1.1</td>
<td>1.1 to 1.2</td>
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<tr>
<td>Insulin (mIU/L)</td>
<td>148</td>
<td>18.5</td>
<td>17.0 to 20.4</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>146</td>
<td>2.6</td>
<td>2.4 to 2.8</td>
</tr>
<tr>
<td>Platelets (x10⁹/L)</td>
<td>228</td>
<td>230.0</td>
<td>218.7 to 242.3</td>
</tr>
<tr>
<td>PRO-C3 (ng/mL)</td>
<td>237</td>
<td>12.3</td>
<td>11.4 to 13.6</td>
</tr>
<tr>
<td>PRO-C6 (ng/mL)</td>
<td>121</td>
<td>12.3</td>
<td>11.0 to 13.3</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>226</td>
<td>1.6</td>
<td>1.5 to 1.8</td>
</tr>
</tbody>
</table>
PRO-C3 is highly related to the severity of NASH histological features

Kruskal-Wallis test followed by Dunn’s multiple comparison test was carried out
* p < 0.0332, ** p < 0.0021, *** p < 0.0002, **** p < 0.0001.
PRO-C3 is highly related to disease activity and fibrosis severity

* p < 0.0332, ** p < 0.0021, *** p < 0.0002, **** p < 0.0001.

#### p < 0.0001
Investigating the association of ECM formation markers to renal function in a sub-population

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n=121</strong></td>
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<td></td>
</tr>
<tr>
<td>Age (Years)</td>
<td>50</td>
<td>47 to 54</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>38</td>
<td>37 to 41</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>71</td>
<td>67.2 to 74.8</td>
</tr>
<tr>
<td>Diabetic</td>
<td>36%</td>
<td></td>
</tr>
<tr>
<td>eGFR (mL/min per 1.73 m²)</td>
<td>93</td>
<td>90 to 99</td>
</tr>
<tr>
<td>Gender</td>
<td>60% Female</td>
<td></td>
</tr>
<tr>
<td>Hypertensive</td>
<td>44%</td>
<td></td>
</tr>
<tr>
<td>PRO-C3 (ng/mL)</td>
<td>16.09</td>
<td>13.9 to 18.3</td>
</tr>
<tr>
<td>PRO-C6 (ng/mL)</td>
<td>12.3</td>
<td>11.0 to 13.3</td>
</tr>
<tr>
<td>Race</td>
<td>97% Caucasian</td>
<td></td>
</tr>
</tbody>
</table>
PRO-C6 but not PRO-C3 is related to Renal Function

K/DOQI CKD nomenclature (Am J Kidney Dis 2002; 39:S1)

Normal renal function >90mL/min/1.73m²
Mildly impaired renal function (IRF) 90-60mL/min/1.73m²
IRF <59mL/min/1.73m²

Kruskal-Wallis test followed by Dunn’s multiple comparison test was carried out
* p < 0.0332, ** p < 0.0021, *** p < 0.0002, **** p < 0.0001.
• PRO-C3 was highly related to key histology parameters of NASH, fibrosis severity and disease activity. Whereas PRO-C6 showed moderate association.

• PRO-C3 showed no relationship to renal function, however PRO-C6 was highly related to renal function as determined by eGFR.

• PRO-C3 is a non-invasive marker of liver fibrosis and PRO-C6 shows promise as a marker for kidney function.
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Kurume University

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