Disease Progression-Related Markers for Aged Non-Alcoholic Fatty Liver Disease Patients

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Research Article

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Abstract

Background and Purpose

The progression of fibrosis is recently acknowledged as an important phenomenon in the progression of non-alcoholic fatty liver disease (NAFLD). Standard markers reflecting liver fibrosis (e.g., FIB-4 index or NFS) have been shown to increase with age. This study aimed to identify fibrosis progression-related markers that are beneficial even in aged individuals.

Methods

A study population of 98 patients diagnosed with NAFLD (non-alcoholic fatty liver [NAFL], n=25; non-alcoholic steatohepatitis [NASH], n=73). Their fibrosis stages were as follows: stage 1 (n=25), stage 2 (n=35), stage 3 (n=33), stage 4 (n=10). Serum levels of pro-inflammatory and anti-inflammatory cytokines were measured by a multiple enzyme-linked immunosorbent assay. Cytokines, the FIB-4 index, and APRI were analyzed to define the best approach to discriminate advanced NAFLD, even in aged (>65 years old) patients.

Results

The following markers showed significant differences between NAFL and NASH: FIB-4, APRI, IP-10, VEGF, and IL-15. The following markers showed significant differences between stage 1-2 and stage 3-4: FIB-4, APRI, IP-10, VEGF, IL-17, PDGF-BB, and RANTES. The fibrosis stage, FIB-4, APRI, PDGF-BB and RANTES were related to the prognosis. In aged patients, IP-10, GM-CSF, RANTES differed between stage 1-2 and stage 3-4.

Conclusion

FIB-4 or APRI, standard NAFLD or fibrosis progression-related markers were beneficial not only for their correlation with fibrosis but also for predicting the prognosis. However, in aged patients, the correlation diminished and RANTES (a chemokine) showed all correlations and should be regarded as beneficial marker in NAFLD.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is an increasing chronic liver disease that may lead to liver cirrhosis and hepatocellular carcinoma (HCC) \(^1\). The advanced stage of NAFLD is known as non-alcoholic steatohepatitis (NASH), while simple fatty liver is known as non-alcoholic fatty liver (NAFL). In recent years, studies on the pathogenesis of NAFLD have revealed that it is associated with multiple parallel hits
from factors such as lipids, inflammatory cytokines, oxidative stress, ER stress, insulin resistance \(^2\). Given that NAFLD patients represent a heterogeneous population, the main disease progression-related pathway in each patient is often difficult to define. Risk stratification is necessary because NAFLD is a progressive disease. The condition of NAFLD, is defined by two approaches: the diagnosis of NAFL or NASH, and the diagnosis of the activity grade and fibrosis stage. The diagnosis of NASH or the activity grade and fibrosis stage requires the pathological examination of a liver biopsy specimen. However, liver biopsy is time-consuming and is associated with a risk of mortality and has the potential for sampling errors \(^3\). Advanced liver fibrosis has been shown to predict worse survival, which should be precisely and quickly evaluated \(^4\).

Recently, non-invasive clinical biological markers or radiological examinations have been employed to predict advanced stages of NAFLD. As clinical biological markers, formulas with standard liver fibrosis-related factors are widely used. The FIB-4 index \(((\text{AST} \times \text{age}) / (\text{platelet count} \times \sqrt{\text{ALT}}))\), NAFLD Fibrosis Score (NFS) \((-1.675 + 0.037 \times \text{age} + 0.094 \times \text{BMI} + 1.13 \times \text{impaired fasting glycemia} / \text{diabetes} [\text{yes}=1, \text{non}=0])\), and APRI \((\text{AST} / \text{upper limit of normal range of AST} / \text{platelet} \times 100)\) are markers that can be simply calculated from standard laboratory data and which show acceptable correlations with histological liver fibrosis and the prognosis \(^5\). The FIB-4 index and NFS are recommended by the EASL-EASD-EASO Clinical Practice Guidelines for ruling out advanced fibrosis \(^6\). However, both of these scores include age in their formulas; thus, care is required in the evaluation. In addition, BMI is included in the NFS. The distribution of BMI is different in different areas: obesity (BMI>30) is relatively prevalent in Western countries, while it is relatively rare in East Asian countries such as Japan. To overcome these disadvantages, other markers that are independent of factors that reflect the clinical status (e.g., age or BMI) are desired.

Immune responses and inflammation are known to be involved in metabolic diseases, including NASH \(^7\). Liver and adipose tissue-derived cytokines are known to promote the progression of metabolic disease. Even in simple fatty liver, macrophage infiltration and macrophage attractant chemokine CCL2 expression are significantly increased \(^8\). Neutrophils are also accepted to be involved in progression of NAFLD. The neutrophil-to-lymphocyte ratio (NLR) is a significant markers for the diagnosis of advanced NAFLD \(^9\). Dendritic cells have also been shown to be involved in the progression of NAFLD progression, while their effect is complex, as pro-inflammatory data and anti-inflammatory data have both been reported \(^7\). T cells are also involved in the progression of NAFLD. In advanced NAFLD, CD4 (+) and CD8 (+) T cell infiltration increases, and inflammatory cytokines, such as IL-6 or IL-8, are also increased \(^8\). Serum cytokine levels do not directly reflect the liver inflammatory status, but can indicate the final balance of these immune responses. Several serum cytokines are recognized as important markers for differentiation of the stages of NAFLD \(^10\).

The objective of the present study is to investigate the effectiveness of standard predictive markers of the NAFLD stage and the profile of multiple cytokines to differentiate between progressive and non-progressive NAFLD patients, including aged patients.
Materials And Methods

Patients

Ninety-eight patients with NAFLD (NAFL, n=25; NASH, n=73) who were diagnosed by liver biopsy at Okayama University Hospital were enrolled. The diagnostic system reported by Matteoni et al. was adopted to diagnose NASH \(^\text{11}\). The METAVIR scoring system was used to analyze the activity grade and stage of liver fibrosis in patients. Serum assays were also conducted for 20 healthy donors. The patients were diagnosed as not having cancer and were negative for hepatitis B and hepatitis C viral markers and autoantibodies. The baseline characteristics of the patients are summarized in Table 1a. The prognosis of the patients was defined based on additional events after liver biopsy (e.g., cirrhosis to death, cirrhosis to hepatocarcinogenesis, non-cirrhotic condition to symptomatic cirrhosis diagnosed by emergence of varix or ascites).

Informed consent

was obtained from each patient included in the study, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the Ethics Committee of Okayama University Hospital.

Blood sample collection and preparation

Fasting blood samples were collected from the patients. The serum was collected on the next day of admission or at the outpatient clinic, meaning that no intervention was performed before sample collection. The serum aliquots were stored at -30°C until the analysis.

Evaluation of standard noninvasive NASH diagnostic formulas

FIB-4 and APRI were evaluated for the differential diagnostic power of NAFL and NASH, stage 1-2 and 3-4. The FIB-4 index and APRI were calculated using the original reported formulas \(^\text{12,13}\).

Multiple cytokine assays

Measurement of multiple cytokines was performed using a BioPlex 200 System (Bio-Rad Laboratories, Hercules, CA, USA) in accordance with the manufacturer’s protocols. The assay was a Bio-Plex Pro Human Cytokine Grp 1 Panel 27-Plex, which targets IL-1\(\beta\), IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, basic fibroblast growth factor (FGF), eotaxin, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage CSF (GM-CSF), interferon (IFN)-\(\gamma\), IP-10, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1\(\alpha\), MIP-1\(\beta\), platelet-derived growth factor subunit B (PDGF-BB), regulated upon activation normal T-cell expressed and secreted (RANTES), tumor necrosis factor (TNF)-\(\alpha\), and vascular endothelial growth factor (VEGF). Samples were tested in duplicate, and the median values were used for further analyses.
Statistical analysis

Statistical comparisons were performed using JMP version 14.0.0 (SAS Institute, Cary, NC, USA). The Wilcoxon rank-sum test was used to compare continuous data, and the chi-squared test was used to compare categorical data. The log-rank test was used for the additional event analysis. P values of <0.05 were considered statistically significant.

Results

Clinical characteristics of the patients according to NAFL vs. NASH

NASH patients were older, with lower serum albumin, lower platelet counts, higher prothrombin time international ratio (PT-INR), higher AST/ALT, and higher homeostasis model assessment as an index of insulin resistance (HOMA-IR) values in comparison to NAFL patients. All NAFL patients showed lower histological activity and fibrosis scores. NASH patients were grouped through the histological analysis (Table 1a).

Clinical characteristics of the patients according to fibrosis stages

Patients with advanced fibrosis were older, with lower serum albumin, lower platelet counts, higher PT-INR, higher AST, and lower triglyceride levels in comparison to patients with mild fibrosis (Table 1b). In addition, advanced fibrosis patients showed higher histological activity in comparison to patients with less advanced fibrosis.

Different patterns of serum cytokine concentrations in NAFL and NASH

As shown in Figure 1a, FIB-4 and APRI showed strong power to discriminate NAFL and NASH. Of the 27 cytokines that were measured, two cytokines; IP-10 and IL-15 were higher in NASH than in NAFL. In contrast, VEGF was decreased in NASH. Other cytokines, such as those representatively shown in the figure (IL-17, PDGF-BB, and RANTES) were equivalent in both NAFL and NASH.

Different patterns of serum cytokine concentrations in mild and advanced fibrosis

FIB-4 and APRI showed good correlation with the progression of the fibrosis stage (Figure 1b). Of the measured cytokines, the level of IP-10 in patients with advanced stage fibrosis was higher than that in patients with mild fibrosis, while the levels of VEGF, IL-17, PDGF-BB, and RANTES were lower in advanced stage patients. IL-15, which differed between NAFL and NASH, did not differ to a statistically significant extent between stage 1-2 and stage 3-4.
Clinical course of patients after liver biopsy

Patients with NASH or advanced fibrosis showed no worsening of mortality. Patients with NASH showed no increase of additional events; however, patients with advanced fibrosis showed more frequent additional events than the others (Figure 2a). These data suggested that advanced fibrosis is more important for their prognosis than the diagnosis of NASH in our cohort.

Cytokine patterns and the clinical course of patients after liver biopsy

The clinical course of the patients was compared depending on the titers of FIB-4, APRI, and selected cytokines (Figure 2b). Patients with high FIB-4 and APRI showed a worse clinical course. Patients with low PDGF-BB and RANTES showed a worse clinical course, while other cytokines (e.g., IP-10, VEGF, IL-15, and IL-17) showed no such correlation with the clinical course. The cytokines useful for dividing NASH and NAFL showed no impact on the clinical course after liver biopsy.

Standard laboratory data and cytokines in NAFL vs. NASH and stage 1-2 vs. 3-4 in young patients

In young patients (<65 years), the FIB-4 index and APRI in the NASH group were higher in comparison to the NAFL group, and they were higher in stage 3-4 than in stage 1-2 (Table 2a). IP-10 was increased in NASH and in stage 3-4, while PDGF-BB, RANTES, and VEGF were decreased in stage 3-4.

Standard laboratory data and cytokines in NAFL vs. NASH and stage 1-2 vs. 3-4 in aged patients

In aged patients (≥65 years), the FIB-4 index and APRI values did not differ to a statistically significant extent between NASH and NAFL, or between stage 1-2 and stage 3-4 (Table 2b). On the other hand, in stage 3-4, IP-10 was increased in comparison to stage 1-2, while GM-CSF and RANTES were obviously decreased in stage 3-4.

Discussion

In this study, FIB-4 and APRI, standard indexes related to the progression of fibrosis showed good correlation with NASH, advanced fibrosis, and disease progression. Although several cytokines showed the same tendency, several cytokines showed the opposite pattern, with as lower values in advanced fibrosis patients. The prognosis was correlated with advanced fibrosis but not with NASH. Although FIB-4 and APRI also showed good correlation with the prognosis, this correlation disappeared in aged patients. PDGF-BB and RANTES, fibrosis related cytokines, showed good correlation with the prognosis. In aged patients, high IP-10, low GM-CSF and low RANTES predicted the progression of fibrosis. RANTES was the only value that showed good correlation with the prognosis in relation to the progression of fibrosis in the overall study population and in aged patients.
Recently, advanced fibrosis has been shown to be a strong prognostic factor, that is even stronger than the diagnosis of NASH. A meta-analysis revealed that NAFLD patients with fibrosis had increased all-cause mortality as the stage progressed: stage 1 (mortality rate ratio (MRR) to stage 0: 1.58), stage 2 (MRR: 2.52), stage 3 (MRR: 3.48), stage 4 (MRR: 6.40). Of course this study showed a stronger effect of fibrosis on the risk of liver-related mortality: stage 1 (MRR: 1.41), stage 2 (MRR: 9.57), stage 3 (MRR: 16.69), stage 4 (MRR: 42.30). Our present study also showed that advanced fibrosis is a risk factor for additional events after liver biopsy, while the diagnosis of NASH had no effect. This is consistent with previous reports.

The prediction of the fibrosis stage via non-invasive markers, such as serological markers or in combination with the clinical status is important, and several markers have been shown to be effective. FIB-4 and NFS are easy to calculate and are widely used in screening for liver fibrosis; however, as age is involved in their calculation, the specificity decline as 35% for FIB-4 and 20% for NFS. Conversely, as these markers include age, they have been shown to be evaluable for predicting the prognosis, when categorized as liver-related mortality or overall mortality. In our present analysis, high FIB-4 and APRI were correlated with a higher frequency of additional events. Of the measured cytokines, inflammatory response-related cytokines, such as IP-10 showed no correlation with the prognosis, while fibrosis-related cytokines, PDGF-BB and RANTES, were predictive of the prognosis.

PDGF plays an outstanding role in the activation of hepatic stellate cells (HSCs). The PDGF-B mRNA expression has been shown to increase in the early stage of HSC activation; this increase is quickly followed by a marked decrease. Immunohistochemical staining of PDGF-BB protein and the analysis of the mRNA expression revealed that they were expressed in portal areas and perisinusoidal cells where myofibroblast-like cells were seen in liver specimens of chronic hepatitis patients. Based on the hepatic expression of PDGF in chronic hepatitis with fibrous expansion, serum PDGF-BB should be correlated with the progression of liver fibrosis; however, the results are confusing. One report showed that serum PDGF-BB increased as alcoholic liver disease progressed, while in chronic hepatitis B patients, the serum PDGF-BB level was negatively correlated with the fibrosis stage and the level in chronic hepatitis C patients was lower than that in healthy subjects. The reason why the serum PDGF-BB levels decrease as chronic hepatitis progresses can be explained as follows. One explanation is that the activation of the PDGF system is strongest in an early stage of fibrosis and declines afterwards, leading to lower serum levels in advanced-stage liver fibrosis. Another explanation is due to the decrease of platelets in advanced liver fibrosis, because the platelets produce PDGF-BB.

Another fibrosis-related marker, RANTES, also called CC chemokine ligand 5 (CCL5) directly activates proinflammatory M1 macrophage polarization and impedes M2 polarization. In patients with HBV-related chronic hepatitis and cirrhosis, the serum RANTES levels of patients with moderate-to-severe hepatitis patients were higher in comparison to patients with mild hepatitis, while the levels in patients with cirrhosis were lower than those in patients with chronic hepatitis. Immunohistological staining of RANTES revealed positivity in the cytoplasm of hepatocytes, while this was diminished in damaged
hepatocytes and fibrous bands of cirrhosis tissue, likely in line with the serum quantity data. The results of our present analysis with NAFLD seem to be in line with the previous results from chronic hepatitis and cirrhosis type B. RANTES showed a good correlation with the progression of fibrosis, even in aged patients, suggesting the superiority of this marker as for defining advanced NAFLD.

Although the above-mentioned fibrosis-related markers showed good correlation with the prognosis and stages of fibrosis in aged patients, markers used to discriminate between NAFL and NASH, such as IP-10, VEGF and IL-15, showed no such correlations. IP-10 is a proinflammatory type 1 helper T (Th1) cell-related marker, that is also referred to as interferon-gamma induced protein of 10 kD or C-X-C motif chemokine ligand 10 (CXCL10), and which has already been shown to be an effective marker for discriminating NASH from NAFL and advanced chronic hepatitis C \(^{10}\). However, IP-10 did not show a good correlation with the prognosis, although it showed a good correlation in patients with advanced fibrosis, even in aged patients.

In conclusion, RANTES is our most recommended non-invasive marker as it correlated with advanced fibrosis stages in young and aged patients and could predict their prognosis. It should be emphasized that FIB-4 and APRI, easily calculated fibrosis-related markers, are also prognostic factors. However, in aged patients, their power is diminished. RANTES showed a good correlation with all of our present results. Further studies, in larger study populations should be performed to confirm the results.

**Declarations**

**Acknowledgements**

The authors would like to thank Asuka Maeda for performing the multiplex ELISA experiments and immunohistochemical staining at our institute.

**Ethics approval and consent to participate**

This study was approved by the ethics committee of Okayama University Hospital. The project number is KEN 1603-026.

Informed consent was obtained from each patient included in the study, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in approval by the ethics committee at Okayama University Hospital. After obtaining the patients' written informed consent, a detailed medical questionnaire was completed by the doctors.

**Consent for publication**

Not applicable.

**Competing interests**
None of the authors have any competing interests.

**Funding**

This work received no funding support.

**Authors’ contributions**

All authors have read and approved the manuscript.

Kosaku Morimoto: Participated in the actual performance of the experiments, data analysis, and writing of the paper

Yasuto Takeuchi, Akinobu Takaki: Participated in the actual performance of the experiments, data analysis, and writing of the paper


Hiroyuki Okada: Participated in the actual performance of writing the paper.

**Acknowledgements**

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**Availability of data and materials**

The datasets analyzed during the current study are not publicly available due to the clinical data are included but are available from the author Akinobu Takaki on reasonable request.

**References**


Tables
Table 1a. Baseline clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>All NAFLD</th>
<th>NAFL</th>
<th>NASH</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>46.5 (40.3-49.0)</td>
<td>54.5 (17-78)</td>
<td>42 (19-66)</td>
<td>56 (17-78)</td>
<td>0.018*</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>55</td>
<td>53</td>
<td>56</td>
<td>44</td>
<td>0.293</td>
</tr>
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<td>BMI</td>
<td>n.t.</td>
<td>27 (14.3-40.7)</td>
<td>26.1 (14.3-36.1)</td>
<td>27.6 (16.7-40.7)</td>
<td>0.347</td>
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<td>Albumin (g/dl)</td>
<td>n.t.</td>
<td>4.4 (3.1-5.1)</td>
<td>4.6 (3.2-5.0)</td>
<td>4.3 (3.1-5.1)</td>
<td>0.021*</td>
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<td>Platelet (/μl)</td>
<td>n.t.</td>
<td>22.6 (6.7-284)</td>
<td>25.6 (10.1-284)</td>
<td>21.8 (6.7-79.1)</td>
<td>0.004*</td>
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<td>T. Bil (mg/dl)</td>
<td>n.t.</td>
<td>0.65 (0.2-6.04)</td>
<td>0.67 (0.31-1.23)</td>
<td>0.65 (0.2-6.04)</td>
<td>0.383</td>
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<td>PT-INR</td>
<td>n.t.</td>
<td>0.97 (0.83-1.47)</td>
<td>0.92 (0.85-1.15)</td>
<td>0.98 (0.83-1.47)</td>
<td>0.002*</td>
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<tr>
<td>AST (U/l)</td>
<td>n.t.</td>
<td>51.5 (0.14-201)</td>
<td>33 (0.14-81)</td>
<td>61 (21-201)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>n.t.</td>
<td>66.5 (14-452)</td>
<td>50 (16-128)</td>
<td>75 (14-452)</td>
<td>0.024*</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>n.t.</td>
<td>159.5 (32-687)</td>
<td>169 (70-687)</td>
<td>154 (32-392)</td>
<td>0.310</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>n.t.</td>
<td>3.44 (0.55-47.56)</td>
<td>1.80 (0.55-47.56)</td>
<td>3.95 (1.32-30.88)</td>
<td>&lt;0.001*</td>
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<tr>
<td>FIB-4 index</td>
<td>n.t.</td>
<td>1.69 (0.007-13.4)</td>
<td>0.7 (0.007-5.2)</td>
<td>2.1 (0.31-13.4)</td>
<td>&lt;0.001*</td>
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<tr>
<td>APRI</td>
<td>n.t.</td>
<td>0.74 (0.002-4.08)</td>
<td>0.40 (0.002-1.56)</td>
<td>0.90 (0.17-4.08)</td>
<td>&lt;0.001*</td>
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</tbody>
</table>

Liver biopsy finding

<table>
<thead>
<tr>
<th></th>
<th>n.t.</th>
<th>Control</th>
<th>All NAFLD</th>
<th>NAFL</th>
<th>NASH</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>activity grade (1; 2-3)</td>
<td>n.t.</td>
<td>70; 28</td>
<td>25; 0</td>
<td>45; 28</td>
<td>&lt;0.001*</td>
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<tr>
<td>fibrosis stage (1-2; 3-4)</td>
<td>n.t.</td>
<td>59; 39</td>
<td>25; 0</td>
<td>34; 39</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Matteoni (1-2; 3-4)</td>
<td>n.t.</td>
<td>25; 73</td>
<td>25; 0</td>
<td>0; 73</td>
<td>&lt;0.001*</td>
<td></td>
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Table 1b. Clinical characteristics according to stage of fibrosis

<table>
<thead>
<tr>
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<th>Stage 1-2</th>
<th>Stage 3-4</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>47 (17-78)</td>
<td>60 (30-75)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>54.2</td>
<td>35.9</td>
<td>0.073</td>
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<tr>
<td>BMI</td>
<td>27.5 (14.3-40.7)</td>
<td>26.9 (19.3-38.4)</td>
<td>0.360</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.5 (3.2-5.1)</td>
<td>4.2 (3.1-4.8)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Platelet (×10^12)</td>
<td>25.2 (10.1-284)</td>
<td>15.7 (6.7-30.5)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>T. Bil (mg/dl)</td>
<td>0.65 (0.2-1.58)</td>
<td>0.71 (0.34-6.04)</td>
<td>0.083</td>
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<tr>
<td>PT-INR</td>
<td>0.95 (0.83-1.15)</td>
<td>1.01 (0.9-1.47)</td>
<td>&lt;0.001*</td>
</tr>
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<td>AST (U/l)</td>
<td>47 (0.14-201)</td>
<td>51 (21-194)</td>
<td>0.034*</td>
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<tr>
<td>ALT (U/l)</td>
<td>69 (16-452)</td>
<td>58 (14-159)</td>
<td>0.250</td>
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<tr>
<td>Triglyceride (mg/dl)</td>
<td>174 (32-687)</td>
<td>126 (43-392)</td>
<td>0.011*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.09 (0.55-47.6)</td>
<td>3.58 (1.32-22.0)</td>
<td>0.052</td>
</tr>
<tr>
<td>FIB-4 index</td>
<td>0.89 (0.0072-5.2)</td>
<td>2.57 (0.94-13.4)</td>
<td>&lt;0.001*</td>
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<tr>
<td>APRI</td>
<td>0.56 (0.002-2.54)</td>
<td>1.02 (0.36-4.08)</td>
<td>&lt;0.001*</td>
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</tbody>
</table>

Liver biopsy finding

- Activity grade (1-2-3): 48; 11 vs. 22; 17, p = 0.006*
- Fibrosis stage (1-2; 3-4): 59; 0 vs. 0; 39, p < 0.001*
- Matteoni (1-2; 3-4): 25; 34 vs. 0; 39, p < 0.001*

Table 2a. Standard laboratory data and cytokines in NAFL vs. NASH and Stage 1-2 vs. Stage 3-4 in patients <65 years of age

<table>
<thead>
<tr>
<th></th>
<th>NAFL</th>
<th>NASH</th>
<th>p</th>
<th>Stage 1-2</th>
<th>Stage 3-4</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIB-4 index</td>
<td>0.70 (0.42-1.18)</td>
<td>1.70 (0.94-3.08)</td>
<td>&lt;0.001*</td>
<td>0.77 (0.45-1.18)</td>
<td>2.55 (1.88-3.84)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>APRI</td>
<td>0.38 (0.22-0.55)</td>
<td>0.88 (0.66-1.38)</td>
<td>&lt;0.001*</td>
<td>0.54 (0.29-0.76)</td>
<td>1.14 (0.81-1.61)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>IP-10</td>
<td>1013 (617-1222)</td>
<td>1330 (880-1712)</td>
<td>0.015*</td>
<td>1055 (684-1420)</td>
<td>1536 (970-1930)</td>
<td>0.006*</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>26.3 (16.3-55.9)</td>
<td>33.4 (16.9-57.9)</td>
<td>0.727</td>
<td>30.8 (16.4-53.7)</td>
<td>36.3 (16.0-59.0)</td>
<td>0.763</td>
</tr>
<tr>
<td>PDGF-BB</td>
<td>4367 (3344-6277)</td>
<td>4045 (2119-7489)</td>
<td>0.536</td>
<td>4906 (3376-6347)</td>
<td>2796 (1517-5151)</td>
<td>0.012*</td>
</tr>
<tr>
<td>RANTES</td>
<td>12068 (10793-13447)</td>
<td>11837 (9689-14578)</td>
<td>0.440</td>
<td>12266 (11000-13663)</td>
<td>10425 (9271-12303)</td>
<td>0.005*</td>
</tr>
<tr>
<td>VEGF</td>
<td>153 (104-218)</td>
<td>120 (80-281)</td>
<td>0.212</td>
<td>147 (116-218)</td>
<td>89 (68-155)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Table 2b. Standard laboratory data and cytokines in NAFL vs. NASH and Stage 1-2 vs. Stage 3-4 in patients ≥65 years of age

<table>
<thead>
<tr>
<th></th>
<th>NAFL</th>
<th>NASH</th>
<th>p</th>
<th>Stage 1-2</th>
<th>Stage 3-4</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIB-4 index</td>
<td>2.13 (1.94-2.31)</td>
<td>2.61 (2.11-4.20)</td>
<td>0.256</td>
<td>2.28 (1.91-2.96)</td>
<td>2.90 (2.30-5.18)</td>
<td>0.069</td>
</tr>
<tr>
<td>APRI</td>
<td>0.81 (0.49-1.13)</td>
<td>1.00 (0.60-1.34)</td>
<td>0.614</td>
<td>0.74 (0.60-1.18)</td>
<td>1.01 (0.67-1.39)</td>
<td>0.226</td>
</tr>
<tr>
<td>IP-10</td>
<td>1976 (1957-1998)</td>
<td>1196 (943-2134)</td>
<td>0.614</td>
<td>1050 (901-1697)</td>
<td>2047 (1146-3212)</td>
<td>0.041*</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>45.2 (29.4-61.0)</td>
<td>40.9 (31.0-102.7)</td>
<td>0.750</td>
<td>78.7 (42.2-126.2)</td>
<td>32.5 (27.2-41.2)</td>
<td>0.020*</td>
</tr>
<tr>
<td>PDGF-BB</td>
<td>3841 (3065-4616)</td>
<td>2750 (466-4757)</td>
<td>0.449</td>
<td>3065 (449-4697)</td>
<td>2750 (1924-5099)</td>
<td>0.705</td>
</tr>
<tr>
<td>RANTES</td>
<td>15149 (11174-11924)</td>
<td>10589 (10086-12650)</td>
<td>0.308</td>
<td>11924 (10616-14606)</td>
<td>10431 (9870-10940)</td>
<td>0.027*</td>
</tr>
<tr>
<td>VEGF</td>
<td>431 (232-629)</td>
<td>79 (54-112)</td>
<td>0.043*</td>
<td>84 (60-204)</td>
<td>78 (46-223)</td>
<td>0.762</td>
</tr>
</tbody>
</table>
Figure 1

Candidate markers to discriminate NAFL and NASH or fibrosis stage 1-2 and 3-4.
The titer of simple clinical markers of the progression of liver fibrosis progression (FIB-4 and APRI) and the concentration of cytokines were investigated to discriminate (a) NAFL and NASH or (b) fibrosis stage 1-2 and 3-4. (a) Both FIB-4 and APRI were effective for discriminating NAFL and NASH. Of the measured cytokines, IP-10 and IL-15 were higher in NASH than in NAFL. In contrast, VEGF was lower in NASH than in NAFL. (b) FIB-4 and APRI were also effective for discriminating stage 1-2 and stage 3-4. IP-10 was higher in stage 3-4 than in stage 1-2, while VEGF, IL-17, PDGF-BB, and RANTES were lower in stage 3-4 than in stage 1-2. Other cytokines showed no significant difference between these clinical stages.

* p<0.05
Candidate markers for predicting the clinical outcome.

Simple clinical markers of the progression of liver fibrosis (FIB-4 and APRI) and cytokine concentrations were investigated to predict clinical outcomes. The prognosis of the patients was defined as an additional event after liver biopsy (cirrhosis to death, cirrhosis to hepatocarcinogenesis, non-cirrhotic...
chronic liver disease to emergence of varix or ascites). (a) The prognosis of the patients with NAFL vs. NASH and fibrosis stage 1-2 vs. 3-4. (b) FIB-4, APRI and cytokines were investigated to determine their prognostic ability. FIB-4, APRI and cytokines PDGF-BB and RANTES were considered useful for predicting the prognosis. * p<0.05