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Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up

Short title: Mortality and causes of death in NAFLD

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Keywords: cohort study, fatty liver, liver fibrosis, NAFLD activity score, mortality

Abbreviations: NAFLD, Nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; HCC, hepatocellular carcinoma; NAS, NAFLD activity score; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; HR, hazard ratio; CVD, cardiovascular disease.

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Background and rationale for the study: Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease in the Western world, strongly associated with insulin resistance and the metabolic syndrome. Nonalcoholic steatohepatitis, i.e. fatty liver accompanied by necroinflammatory changes, is mostly defined by the NAFLD activity score (NAS). The aim of the current study was to determine disease-specific mortality in NAFLD, and evaluate the NAS and fibrosis stage as prognostic markers for overall and disease-specific mortality.

Methods: In a cohort study, data from 229 well-characterized patients with biopsy-proven NAFLD were collected. Mean follow-up was 26.4 (± 5.6, range 6-33) years. A reference population was obtained from the National Registry of Population, and information on time and cause of death were obtained from the Registry of Causes of Death.

Main results: NAFLD patients had an increased mortality compared with the reference population (HR 1.29, CI 1.04-1.59, p=0.020), with increased risk of cardiovascular disease (HR 1.55, CI 1.11-2.15, p=0.01), hepatocellular carcinoma (HR 6.55, CI 2.14-20.03, p=0.001), infectious disease (HR 2.71, CI 1.02-7.26, p=0.046), and cirrhosis (HR 3.2, CI 1.05-9.81, p=0.041). Overall mortality was not increased in patients with NAS 5-8 and fibrosis stage 0-2 (HR 1.41, CI 0.97-2.06, p=0.07), whereas patients with fibrosis stage 3-4, irrespective of NAS, had increased mortality (HR 3.3, CI 2.27-4.76, p<0.001).

Conclusions: NAFLD patients have increased risk of death, with a high risk of death from cardiovascular disease and liver-related disease. The NAS was not able to predict overall mortality, whereas fibrosis stage predicted both overall and disease-specific mortality.
Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease in the Western world, strongly associated with insulin resistance and the metabolic syndrome.\(^1\)\(^-\)\(^3\) NAFLD encompasses a spectrum of histopathological conditions, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis, with risk of hepatocellular carcinoma (HCC).\(^4\) The prevalence of NAFLD in the general population is in the range of 20-40 % to a large extent influenced by the occurrence of obesity,\(^2\)\(^,\)\(^5\) and the prevalence of NASH could be as high as 12 %.\(^6\) NASH is considered the more aggressive part of the NAFLD spectrum associated with progressive fibrosis as well as higher risk of cardiovascular disease.\(^7\)\(^,\)\(^8\) Today, NAFLD is the third most common cause for need of liver transplantation, and on the trajectory of becoming the most common cause.\(^9\)\(^,\)\(^10\)

The prognosis for the individual patient with NAFLD is highly variable. Worst prognosis has been described in patients with NASH, i.e. fatty liver accompanied by necroinflammatory changes such as lobular inflammation and hepatocellular ballooning,\(^11\) features that can only be diagnosed after performing liver biopsy. However, over the years several definitions of NASH have been used.\(^12\) In recent years the NAFLD activity score (NAS) has gained wide acceptance in defining NASH although it was never intended to replace the pathologist’s decision on whether NASH was present or not.\(^13\) The NAS is the unweighted sum of steatosis, ballooning, and lobular inflammation. Despite the high variability of at least two of the three components of NAS, reported already in the original publication\(^13\), and limited data on the ability of NAS to predict disease outcome, NAS is recommended to be used to define and quantify disease activity in clinical trials of NAFLD/NASH.\(^14\)
Because of the very high prevalence of NAFLD in the general population and the variable prognosis in individual patients there is a need to assess the prognostic value of liver histology to predict disease outcome. Recently fibrosis stage was suggested to predict liver-specific mortality more reliably than NAS,\textsuperscript{15} furthermore the ability of NAS to predict progression of fibrosis has been shown to be poor.\textsuperscript{16, 17}

The aim of the current study was to determine if long-term disease specific mortality in NAFLD was increased compared to the general population, and to evaluate the ability of the NAS and fibrosis stage to serve as prognostic markers for overall and disease-specific mortality.

**Patients and Methods**

In this cohort study, we performed an extended follow-up in 229 well-characterized patients with biopsy-proven NAFLD from two centers in Sweden previously described in two publications.\textsuperscript{21, 22} Between 1980 and 1993, 444 patients with persistently (>6 months) elevated serum alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) with or without elevation of alkaline phosphatase were included in three studies investigating the etiology of elevated liver function tests in Sweden.\textsuperscript{18-20} A diagnostic work-up was performed including physical examination, laboratory investigation, and liver biopsy. Patients with a confirmed diagnosis of NAFLD were included in the present study.

Exclusion criteria were symptoms or clinical signs of advanced liver disease at baseline, liver disease of other etiology, such as alcoholic or drug induced liver disease,
autoimmune liver disease, viral hepatitis, cholestatic, or genetic liver disease. All patients were originally enrolled by an experienced hepatologist and great deal of effort was put into uncovering overconsumption of alcohol at baseline. Patients with a previous diagnosis of diabetes mellitus or with fasting glucose > 126 mg/dL were defined as diabetics. Hypertension was defined as systolic blood pressure >130/85 mmHg or requiring treatment. Cardiovascular disease was defined as previously diagnosed coronary heart disease, cerebrovascular disease or peripheral arterial disease. Hypertriglyceridemia was defined as fasting triglyceride > 140 mg/dL, and hypercholesterolemia was defined as total cholesterol > 240 mg/dL. As part of this follow-up study all medical records were reviewed with special attention to information on alcohol consumption, and if viral hepatitis had been diagnosed during follow-up. Patients with a reported episode of overconsumption of alcohol or a consumption of ≥140 g per week were excluded. A flow chart of the patient inclusion process is presented in Figure 1.

**Liver biopsy and histopathological re-evaluation.**

Liver biopsies were performed on an outpatient basis using a 1.4- or 1.6-mm Menghini-needle. Of the 229 liver biopsies from NAFLD patients, 155 were re-evaluated by an experienced liver pathologist (R.H.) blinded to patient details and outcomes. The old slides were usually well preserved but in those with fading staining new sections and staining were performed, six biopsies were excluded from sub-group analyses because of poor staining. Seventy four liver biopsies were not available for reassessment but had previously been reassessed by an experienced liver pathologist as part of a prior follow-up study. There was a low reproducibility ($\kappa = 0.062$) of hepatocellular ballooning and lobular inflammation between the two pathologists. Therefore, these 74 patients were
excluded from the analyses of NAS. However, they were still included in the analyses of fibrosis stage, as the agreement on fibrosis stage between the two pathologists was substantially higher ($\kappa = 0.73$).

Liver histology was scored in accordance with the system developed by Kleiner et al, and the NAS was calculated as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3), and hepatocellular ballooning (0-2).\textsuperscript{13}

**Data collection.**

At the time of liver biopsy extensive clinical and laboratory data were collected including a full medical history and physical examination. All subjects had blood drawn after an overnight fasting for analysis of complete blood count, AST, ALT, alkaline phosphatase, gamma glutamyl transferase, bilirubin, total cholesterol, triglycerides, plasma protein electrophoresis including albumin, $\alpha_1$-antitrypsin, ceruloplasmin, and immunoglobulins. Moreover, blood was obtained for detection of hepatitis B surface antigen, antinuclear antibodies, smooth muscle antibodies, and mitochondrial antibodies. At the time of the baseline studies, testing for Hepatitis C was not available. In two previous follow-up studies, 113 of the included NAFLD patients were tested for Hepatitis C, and none tested positive.\textsuperscript{21,22} Three patients were excluded after reviewing their medical charts because they had tested positive for Hepatitis C. Body mass index (BMI) was calculated using the formula: weight (kilograms)/height$^2$ (m$^2$).

All residents in Sweden have a unique national registration number issued by the Swedish Tax Agency. Information on place of birth, emigration, and death is recorded annually in the Register of Population and Population changes and each individual was
identified with their national registration number. Therefore all NAFLD patients could be followed longitudinally. The national registration number is recorded at all visits to health care facilities and in census registers making linkage possible. A reference population was obtained from Statistics Sweden. For each NAFLD patient ten residents matched for date of birth and sex living in the same county were selected. Information on time of and cause of death for both patients and the reference population were obtained from the Registry of Causes of Death. Causes of death were coded according to the International Classifications of Diseases versions 8, 9 and 10, depending on time of death.

**Statistical Analysis**

Continuous variables are presented as mean (standard deviation), and categorical variables are presented as number (percentage). Histopathological agreement was analyzed by the kappa (κ) coefficient. Analyses of mortality risks were done with the proportional hazard model (Cox regression), stratified on matching number. The model was tested for proportionality with Schoenfeld residuals. Survival curves were made according to the Kaplan-Meier method. All statistical analyses were performed with Stata v12.1 (StataCorp LP, College Station TX, USA). A p-value < 0.05 was considered statistical significant.

**Ethical considerations**

This study was approved by the ethical review boards in both Linköping and Stockholm.
Results

Cohort characteristics
The mean age of the 229 NAFLD patients, at time of liver biopsy, was 48.8 ± 12.8 years, with a male predominance (66 %). One hundred and twenty-nine patients (57%) were overweight (BMI > 25 and ≤ 30) and a further 59 patients (26 %) were obese (BMI > 30). Type 2 diabetes mellitus had previously been diagnosed in 31 patients (14 %), of whom 8 (26%) was treated with hypoglycemic drugs, and 22 patients (9.7 %) had manifest cardiovascular disease. Dyslipidemia was common, with 118 patients (52 %) having hypertriglyceridemia (>140mg/dL) and 78 patients (34 %) hypercholesterolemia (>240 mg/dL). ALT levels were more increased than AST levels, 77 ± 44 vs 47 ± 26 U/L. Baseline characteristics of the patient cohort are presented in Table 1.

Liver histology
The original histology reports were available on all 229 patients from the two previous studies. Of these, 149 liver biopsies could be re-evaluated and scored for NAS, whereas 74 biopsies were missing and only scored and analyzed for fibrosis stage based on a previous re-evaluation. Of the 149 patients with complete histological data, 76 patients (49%) had NAS 0-4 and fibrosis stage 0-2, 57 patients (37%) had NAS 5-8 and fibrosis stage 0-2, eight patients (5%) had NAS 0-4 and fibrosis stage 3-4, and finally eight patients (5%) had NAS 5-8 and fibrosis stage 3-4 (Table 2). Of the 74 patients in whom histology were missing for re-evaluation, 65 (86%) had fibrosis stage 0-2 and 11 (14%) had fibrosis stage 3-4.
Causes of death

The cohort was followed for a mean of 26.4 years (± 5.6, range 6-33), or 5400 person years. During follow-up, 96 patients and 786 individuals from the reference population died. In 6 patients and 40 reference individuals no cause of death were provided from the Registry of Causes of Death, which is either due to death in recent time or missing data entry from the reporting clinician. These patients were included in the analysis of overall mortality, but left out in the analysis of disease specific mortality. Four patients emigrated during the study period and were lost to follow-up. These patients still contributed with 31 years of follow-up time to the analysis. Overall mortality in the entire cohort was significantly increased, with a hazard ratio of 1.29 (95 % CI 1.04-1.59, \(p=0.020\)). Causes of death for the 96 patients are listed in Table 3. A survival curve of the entire cohort vs. the reference population is presented in Figure 2a.

Data regarding hazard ratios for the entire cohort and on subgroup levels are presented in Table 4. Increased risks of disease specific causes of death were found for cardiovascular disease (HR 1.55, 95 % CI 1.11-2.15, \(p=0.01\)), hepatocellular carcinoma (HR 6.55, 95 % CI 2.14-20.03, \(p=0.001\)), infections (HR 2.71, 95 % CI 1.02-7.26, \(p=0.046\)) and cirrhosis (HR 3.2, 95 % CI 1.05-9.81, \(p=0.041\)). No increased risks were found for death from non-gastrointestinal malignancies, gastrointestinal malignancy, renal, respiratory or other causes of death.

When excluding patients with diabetes at baseline (\(n=31\)), overall mortality (HR 1.15, 95 % CI 0.91-1.47, \(p=0.248\)) and death from cardiovascular disease (HR 1.32, 95 % CI 0.89-1.96, \(p=0.169\)) were no longer statistically significant. However, death from cirrhosis (HR 3.13, 95 % CI 1.08-9.12, \(p=0.036\)) and hepatocellular carcinoma (HR 4.02,
95 % CI 1.46-11.07, p<0.007) were still significantly higher in NAFLD patients compared with the reference population.

Subgroup analysis
In the subgroup of patients with NAS 0-4 and fibrosis stage 0-2, overall mortality was not increased compared to the reference population (HR 1.13, 95 % CI 0.79-1.60, p=0.51). There were no cases of HCC in this group. Death from events related to cirrhosis was significantly increased (HR 4.86, 95 % CI 1.08-22.0, p=0.04). This was due to death of two patients with cirrhosis, with fibrosis stage 2 at baseline. Neither death from cardiovascular disease, non-gastrointestinal malignancy or other diseases was significantly increased compared to the reference population.

In the subgroup of patients with NAS 5-8 and fibrosis 0-2, overall mortality was not increased (HR 1.41, 95 % CI 0.97-2.06, p=0.07). Death from HCC was significantly increased (HR 15.67, 95 % CI 4.1-59.86, p<0.001). Mortality from CVD was not different from that of the reference population (HR 1.38, 95 % CI 0.72-2.65, p=0.34). There was also an increased risk of death from respiratory diseases, e.g. chronic obstructive pulmonary disease or asthma (HR 3.95, 95 % CI 1.2-13.0, p=0.024).

Apart from 3 deaths from HCC, there were no cases of death from events linked to cirrhosis in this group. All patients with HCC in this group had developed cirrhosis during follow-up, and the fibrosis stages at baseline were 0, 2, and 2, respectively in these patients. Mortality from other diseases was not different compared to the reference population (data not shown). A survival curve of the overall mortality in
patients with NAS 5-8 and fibrosis 0-2 vs. patients with NAS 0-4 and fibrosis 0-2 vs. the reference population is presented in Figure 3.

There were no significant differences regarding any cause of death between the groups NAS 0-4 / fibrosis stage 3-4 and NAS 5-8 / fibrosis stage 3-4. Therefore, the data in this group was pooled and analyzed together. The overall mortality in this group (NAS 0-8, fibrosis stage 3-4) was significantly increased (HR 3.3, 95%CI 2.27-4.76, p<0.001). Death from CVD, HCC, infection and cirrhosis were all significantly increased compared to the reference population according to Table 4. A survival curve of overall mortality in patients with fibrosis stage 3-4 vs. fibrosis stage 0-2 vs. the reference population is presented in Figure 2c.

Discussion

In this large cohort of patients with biopsy proven NAFLD we found an increased mortality compared with a matched reference population, mainly attributed to increased risk of death due to cardiovascular disease and liver-related death. Worst prognosis was found in patients with fibrosis stage 3 or 4 at baseline. Interestingly, patients with high NAS (grade 5-8) without severe fibrosis (stage 0-2) did not have increased mortality compared to the reference population.

The main strengths of the present study are firstly that we present the hitherto longest follow-up time ever documented in patients with NAFLD. Secondly, the diagnosis of NAFLD, NAS and fibrosis stage were in all instances based on liver biopsy, which is considered the “gold standard”. Furthermore, patients were enrolled consecutively and the reason for performing liver biopsy in each individual was to investigate the etiology
of elevated liver function tests and not because severe histopathological hepatic damage was suspected.

Our study has some limitations. Firstly, all patients were initially investigated because of elevated transaminases. Even though it has previously been shown that the entire spectrum of NAFLD can be found in patients with normal liver function tests and that histopathology does not differ between patients with normal and elevated aminotransferases, we cannot rule out that patients with elevated liver function tests have a worse prognosis. Moreover, all patients had been referred for further evaluation to a liver unit, which may open for selection bias. We had no clinical data on the reference population, and were therefore unable to control for important factors such as smoking, metabolic syndrome and use of medication. Since the total numbers of deaths from cirrhosis (n = 5), HCC (n = 5), infectious disease (n = 5) and respiratory diseases (n = 3) were low, the significantly increased risks of disease-specific mortality on subgroup levels should be interpreted cautiously. The finding that the subgroup of patients with NAS 0-4 and fibrosis stage 0-2 did not have an increased overall mortality but an increase in death from events related to cirrhosis is unexpected, but these cases (N=2) occurred in patients with fibrosis stage 2 at baseline, indicating that these patients may had progressed to cirrhosis during follow-up. These two patients had NAS 2 and 4 respectively. Again, the large confidence intervals in this subgroup indicates that the results should be interpreted carefully.

Increased incidence of death from HCC was observed in patients with high NAS without fibrosis, in patients with advanced fibrosis regardless of NAS grade as well as in the entire cohort. Some previous studies have indicated that HCC can arise in both cirrhotic
and non-cirrhotic NAFLD patients.\textsuperscript{24,25} In our study all patients developing HCC had underlying cirrhosis, thereby indicating that HCC is mainly a complication of cirrhosis in NAFLD.

The increased risk of overall mortality and death from cardiovascular disease was not observed when excluding NAFLD patients with diabetes. However, an increased risk of death from liver-related complications was still observed in this group. These data support those from previous studies showing a better prognosis for NAFLD patients without diabetes.\textsuperscript{11} The increased risk of developing liver-related complication without also in the absence of diabetes is interesting. The prevalence of diabetes in the reference populations is not known. If we exclude patients with diabetes solely in the NAFLD cohort the result will be skewed towards a worse prognosis for the reference population, and a potential difference in overall mortality will disappear. This could possibly be an explanation for the loss of increased risk for overall mortality and death from cardiovascular disease after exclusion of diabetics in the NAFLD cohort.

The majority of the male population and one third of females in Sweden are overweight,\textsuperscript{26} and thus NAFLD is likely to be prevalent in many subjects of the reference population. This fact would dilute the effect, creating a risk of underestimating the true hazard ratios for the outcomes in our cohort.

Several follow up studies have shown that cardiovascular disease is the most common cause of death in NAFLD patients.\textsuperscript{27-30} This is in accordance with previous prospective cohort studies of NAFLD diagnosed by ultrasonography.\textsuperscript{31-34} The high cardiovascular mortality in NAFLD could partly be explained by the fact that 9.7% of our patients had
manifest cardiovascular disease at baseline. However, the present study is, together with the previously published studies on the same cohorts,\textsuperscript{21,22} the only studies that show increased risk of death from CVD in a cohort with biopsy-proven NAFLD. On a subgroup level, we found that the risk of death from cardiovascular disease was only increased in patients with advanced fibrosis which could explain why studies using elevated serum alanine aminotransferase as a surrogate marker of NAFLD failed to show increased risk of CVD.\textsuperscript{35} Thus, our results also underline the benign nature of fatty liver disease without severe fibrosis.\textsuperscript{36}

The finding that NAS was not associated with increased risk of death from cirrhosis or cardiovascular disease in patients without fibrosis confirms the results of a recently published study.\textsuperscript{37} In that study which included 320 patients with biopsy-proven NAFLD there was no difference in outcome between patients with and without definite NASH. This observation may have profound impact on the design of clinical trials.\textsuperscript{38} In comparison with the aforementioned cohort, the present study had significantly longer follow-up, and patients were less obese (BMI 28 vs. 33 kg/m\textsuperscript{2}) with relatively few patients with advanced fibrosis (12% vs. 51% with stage 3-4). Therefore, our results more likely represent disease progression in the general population, improving external validity. However, due to the relatively small number of patients in each subgroup the confidence intervals were relatively wide and conclusions must be interpreted cautiously.

The NAS is a composite score.\textsuperscript{13} The subscore of steatosis is graded as 0-3 points, giving a large impact on the total score (0-8). It is possible that steatosis per se is less useful as a prognostic marker compared to ballooning and lobular inflammation, which could
have impact on the ability of the NAS to prognosticate mortality. If so, ballooning and lobular inflammation could in fact be potential prognostic markers for mortality, something this study was not designed to investigate. Moreover, fibrosis stage is not included in the NAS, and given the apparent effect of fibrosis stage on mortality, potential future prognostic scoring systems for NASH should take fibrosis stage into account.

The inability of the NAS to predict liver-related mortality has also been demonstrated by Younossi et al in a recent study on 257 NAFLD patients. The combined results from these studies clearly emphasize the need to validate the histopathological variables associated with progressive disease before they are used as end-points in clinical trials.

Our study demonstrates that in NAFLD, fibrosis stage is a more robust predictor than the NAS of both overall and disease-specific mortality, which is in accordance with a recent publication based on the National Health And Nutrition Examinations Survey (NHANES). The present study is also the first to report that NAFLD patients in general, apart from having increased overall mortality, also have significantly increased risk of dying from cardiovascular disease, as well as from liver-related events including HCC.

In summary, the present study show that NAFLD patients have an increased risk of death compared to a sex and age-matched reference population, with a high risk of death from cardiovascular disease and liver-related disease. NAS, the currently most widely used histologic scoring system for NASH, is not able to predict overall mortality. There is an urgent need of new liver histology scoring systems. Our results indicate that
fibrosis stage is the most useful marker to predict future mortality, in patients with NAFLD.

Acknowledgements

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References


Table 1. Baseline characteristics of the patient cohort [Mean ± SD or n (%)].

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.8 ± 12.8</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>149 (66%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.3 ± 3.7</td>
</tr>
<tr>
<td>Overweight&lt;sup&gt;a&lt;/sup&gt;</td>
<td>129 (57%)</td>
</tr>
<tr>
<td>Obese&lt;sup&gt;b&lt;/sup&gt;</td>
<td>59 (26%)</td>
</tr>
<tr>
<td>Smoker, present</td>
<td>49 (21%)</td>
</tr>
<tr>
<td>Smoker, ex</td>
<td>49 (21%)</td>
</tr>
<tr>
<td>Diabetes&lt;sup&gt;c&lt;/sup&gt;</td>
<td>31 (14%)</td>
</tr>
<tr>
<td>Hypertensive&lt;sup&gt;d&lt;/sup&gt;</td>
<td>130 (57%)</td>
</tr>
<tr>
<td>Manifest cardiovascular disease&lt;sup&gt;e&lt;/sup&gt;</td>
<td>22 (9.7%)</td>
</tr>
<tr>
<td>Hypertriglyceridemia&lt;sup&gt;f&lt;/sup&gt;</td>
<td>118 (52%)</td>
</tr>
<tr>
<td>Hypercholesterolemia&lt;sup&gt;g&lt;/sup&gt;</td>
<td>78 (34%)</td>
</tr>
<tr>
<td>Hypoglycemic treatment</td>
<td>8 (3.5%)</td>
</tr>
<tr>
<td>Anti-hypertensive treatment</td>
<td>64 (28%)</td>
</tr>
<tr>
<td>Lipid-lowering treatment</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>77 ± 44</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>47 ± 26</td>
</tr>
<tr>
<td>AST/ALT ratio</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>64 ± 34</td>
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<tr>
<td>Bilirubin (mg/dL)</td>
<td>0.68 ± 0.37</td>
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<tr>
<td>Albumin (g/dL)</td>
<td>4.3 ± 0.4</td>
</tr>
<tr>
<td>Platelet count (x 10&lt;sup&gt;9&lt;/sup&gt;/L)</td>
<td>233 ± 62</td>
</tr>
<tr>
<td>Prothrombin (INR)</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Ferritin (mikrog/L)</td>
<td>218 ± 300</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>201 ± 171</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>103 ± 41</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>236 ± 59</td>
</tr>
<tr>
<td>Alpha-1-antitrypsin (µmol/L)</td>
<td>24 ± 8.6</td>
</tr>
<tr>
<td>Ceruloplasmin (mg/dL)</td>
<td>34 ± 11</td>
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<tr>
<td>Immunoglobulin G (mg/dL)</td>
<td>1172 ± 233</td>
</tr>
<tr>
<td>Immunoglobulin M (mg/dL)</td>
<td>116 ± 61</td>
</tr>
<tr>
<td>Immunoglobulin A (mg/dL)</td>
<td>250 ± 100</td>
</tr>
<tr>
<td>ANA/SMA/AMA (%)</td>
<td>29/15/0</td>
</tr>
</tbody>
</table>

<sup>a</sup>BMI > 25 kg/m² but ≤ 30 kg/m²; <sup>b</sup>BMI > 30 kg/m²; <sup>c</sup>fasting plasma glucose > 126 mg/dL or previously diagnosed or treated; <sup>d</sup>blood pressure >130/85 or requiring treatment; <sup>e</sup>previously diagnosed coronary heart disease, cerebrovascular disease or peripheral arterial disease; <sup>f</sup>fasting triglycerides > 140 mg/dL; <sup>g</sup>total cholesterol > 240 mg/dL.
Table 2. Histopathological sub-groups in NAFLD patients (n = 149).

<table>
<thead>
<tr>
<th>NAS</th>
<th>Fibrosis stage</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0-2</td>
<td>3-4</td>
<td></td>
</tr>
<tr>
<td>0-4</td>
<td>76 (51 %)</td>
<td>8 (5 %)</td>
<td></td>
</tr>
<tr>
<td>5-8</td>
<td>57 (38 %)</td>
<td>8 (5 %)</td>
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</table>
Table 3. Causes of death in NAFLD patients [n (%)].

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Number of patients (n = 96)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular disease</td>
<td>41 (43%)</td>
</tr>
<tr>
<td>Non-gastrointestinal malignancy</td>
<td>18 (19%)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>5 (5%)</td>
</tr>
<tr>
<td>Infection</td>
<td>5 (5%)</td>
</tr>
<tr>
<td>Gastrointestinal malignancy</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>Endocrine</td>
<td>3 (3%)*</td>
</tr>
<tr>
<td>Respiratory</td>
<td>3 (3%)</td>
</tr>
<tr>
<td>Other</td>
<td>7 (7%)</td>
</tr>
<tr>
<td>Missing</td>
<td>6 (6%)</td>
</tr>
</tbody>
</table>

*All three patients died from complications related to type 2 diabetes.
Table 4. Hazard ratios for causes of death in the entire cohort and in histopathological subgroups compared with the reference population. [HR (95% CI)].

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Entire cohort (n = 229)</th>
<th>P</th>
<th>NAS 0-4, F0-2 (n = 76)</th>
<th>P</th>
<th>NAS 5-8, F0-2 (n = 57)</th>
<th>P</th>
<th>NAS 0-8, F3-4 (n = 16)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall mortality</td>
<td>1.29 (1.04-1.59)</td>
<td>0.020</td>
<td>1.13 (0.79-1.60)</td>
<td>0.511</td>
<td>1.41 (0.97-2.06)</td>
<td>0.072</td>
<td>3.28 (2.27-4.76)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>1.55 (1.11-2.15)</td>
<td>0.01</td>
<td>1.19 (0.65-2.20)</td>
<td>0.557</td>
<td>1.38 (0.72-2.65)</td>
<td>0.335</td>
<td>4.36 (2.29-8.29)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>6.55 (2.14-20.00)</td>
<td>0.001</td>
<td>No outcome</td>
<td>-</td>
<td>15.7 (4.1-59.9)</td>
<td>&lt;0.001</td>
<td>16.9 (1.95-146)</td>
<td>0.01</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>3.2 (1.05-9.81)</td>
<td>0.041</td>
<td>4.86 (1.08-22.0)</td>
<td>0.04</td>
<td>No outcome</td>
<td>-</td>
<td>10.8 (1.38-83.9)</td>
<td>0.023</td>
</tr>
<tr>
<td>Gastrointestinal malignancy</td>
<td>0.60 (0.22-1.64)</td>
<td>0.322</td>
<td>1.26 (0.60-2.65)</td>
<td>0.546</td>
<td>0.54 (0.075-3.96)</td>
<td>0.548</td>
<td>No outcome</td>
<td>-</td>
</tr>
<tr>
<td>Non-gastrointestinal malignancy</td>
<td>1.18 (0.70-1.98)</td>
<td>0.545</td>
<td>1.24 (0.55-2.76)</td>
<td>0.602</td>
<td>0.85 (0.27-2.65)</td>
<td>0.778</td>
<td>No outcome</td>
<td>-</td>
</tr>
<tr>
<td>Infectious disease</td>
<td>2.71 (1.02-7.26)</td>
<td>0.046</td>
<td>3.12 (0.72-13.5)</td>
<td>0.129</td>
<td>2.22 (0.31-16.4)</td>
<td>0.435</td>
<td>13.0 (3.13-54.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Respiratory disease</td>
<td>1.01 (0.31-3.32)</td>
<td>0.979</td>
<td>No outcome</td>
<td>-</td>
<td>3.95 (1.22-13.0)</td>
<td>0.024</td>
<td>No outcome</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: NAS, NAFLD activity score; F, Fibrosis stage; HR; Hazard ratio; CI: confidence interval. NAS is the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3), and hepatocellular ballooning (0-2).
Figure 1. Details of included and excluded patients. HH, hereditary hemochromatosis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; AAT, $\alpha_1$-antitrypsin.

Figure 2a. Survival of entire cohort compared to the reference population.

Figure 2b. Survival of patients with fibrosis stage 0-2 and NAS 0-4, fibrosis stage 0-2 and NAS 5-8, respectively compared to the reference population.

Figure 2c. Survival of patients with fibrosis stage 0-2, and 3-4 compared to the reference population.
Subjects evaluated during 1988-1993 because of chronically Elevated serum aminotransferases and/or alkaline phosphatase

444 subjects

Hepatitis B/C (n = 67)
HH (n = 3)
PBC (n = 14)
PSC (n = 8)
Autoimmune hepatitis (n = 16)
AAT-deficiency (n = 8)
Normal liver biopsy (n = 37)
Pathologic liver biopsy without steatosis (n = 5)
Excessive alcohol consumption\(^a\) (n = 40)
Other (n = 17)

Subjects with NAFLD Included in the present study

229 subjects

96 subjects died during follow-up
133 subjects still alive

\(^a\) Defined as having an episode of excessive drinking or an average alcohol consumption > 140 gram per week
Total number of deaths

Log-rank test: p=0.17

Number at risk

<table>
<thead>
<tr>
<th>Group</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAS&lt;5 &amp; Fibrosis&lt;3</td>
<td>76</td>
<td>70</td>
<td>61</td>
<td>16</td>
</tr>
<tr>
<td>NAS&gt;4 &amp; Fibrosis&lt;3</td>
<td>57</td>
<td>53</td>
<td>39</td>
<td>12</td>
</tr>
<tr>
<td>Controls</td>
<td>2286</td>
<td>2085</td>
<td>1818</td>
<td>387</td>
</tr>
</tbody>
</table>

---

- NAS<5 & Fibrosis<3
- NAS>4 & Fibrosis<3
- Controls
Total number of deaths

Log-rank test: p<0.001

Number at risk

<table>
<thead>
<tr>
<th></th>
<th>Controls 2286</th>
<th>Fibrosis&lt;3 198</th>
<th>Fibrosis&gt;2 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2286</td>
<td>198</td>
<td>25</td>
</tr>
<tr>
<td>10</td>
<td>2085</td>
<td>184</td>
<td>21</td>
</tr>
<tr>
<td>20</td>
<td>1818</td>
<td>156</td>
<td>13</td>
</tr>
<tr>
<td>30</td>
<td>387</td>
<td>28</td>
<td>0</td>
</tr>
</tbody>
</table>

- Controls
- Fibrosis<3
- Fibrosis>2