Non-alcoholic steatofibrosis (NASF) can independently predict mortality in patients with non-alcoholic fatty liver disease (NAFLD)

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ABSTRACT

Background Hepatic fibrosis in patients with non-alcoholic fatty liver disease (NAFLD) independently predicts mortality. Given liver biopsy’s invasive nature, non-invasive method to assess hepatic steatosis and fibrosis provides NAFLD risk stratification algorithm in clinical practice. NAFLD fibrosis score (NFS) is simple and non-invasive predictive model recommended by American Association for the Study of Liver Disease (AASLD) Guideline to identify patients with NAFLD with fibrosis risk. The aim of this study is to assess long-term outcomes of subjects with significant non-alcoholic steatofibrosis (NASF) as established by ultrasound (US) and NFS.

Methods Used National Health and Nutrition Examination Survey (NHANES III) with National Death Index-linked Mortality Files. NAFLD diagnosis established by the presence of moderate to severe hepatic steatosis on US without other causes of chronic liver disease (alcohol consumption <20 gr/day, hepatitis B surface-antigen negative, anti-hepatitis C virus antibody negative, transferrin saturation <50%). Significant hepatic fibrosis was estimated by high NFS (>0.676) and calculated with previously published formula. Subjects with NAFLD and high NFS have significant NASF.

Results NHANES III included 20050 adult participants. 2515 participants complete data and NAFLD with 5.1% (n=129) meeting criteria for significant SF. Subjects with significant SF were older, had higher body mass index, waist circumference and the homeostasis model assessment (HOMA) scores and higher rates of comorbidities (diabetes, congestive heart failure (CHF), stroke; all p<0.001). After median of 207 months of follow-up, overall mortality in NAFLD cohort was 30.0% (n=754). Crude mortality higher in subjects with significant SF (67.4% vs 28.0%, p<0.001). In multivariate survival analysis, predictors of overall mortality included significant SF (adjusted HR (aHR): 1.37; 95% CI 1.07 to 1.76, p=0.01), older age (aHR:1.08; 95% CI 1.07 to 1.09 per year), male gender (aHR:1.44; 95% CI 1.24 to 1.67), black race (aHR:1.24; 95% CI 1.04 to 1.48)), history of hypertension (aHR:1.40; 95% CI 1.20 to 1.64), diabetes (aHR:1.69; 95% CI 1.43 to 2.00), CHF (aHR:1.77; 95% CI 1.38 to 2.61), stroke (aHR:1.84; 95% CI 1.38 to 2.48) and smoking (aHR:1.74; 95% CI 1.47 to 2.07) (all p<0.02). Sensitivity analysis showed that the best association of SF with mortality is higher at NFS threshold of 0.80 (aHR:1.41; 95% CI 1.09 to 1.83, p=0.01).

Conclusions Significant NASF determined non-invasively is an independent predictor of overall mortality. These data should help clinicians to easily risk-stratify patients with NAFLD for close monitoring and treatment considerations in clinical trial setting.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the leading aetiologies of chronic liver disease worldwide, affecting more than a quarter of the general population.1–3 In addition to its clinical impact, NAFLD can also interfere with patients’ quality of life.4–6 The clinicopathological spectrum of NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis (NASH), which can be accompanied by significant hepatic fibrosis and a risk of progression to advanced liver disease.7-9 The ‘gold standard’ for staging hepatic fibrosis is a liver biopsy, which is invasive, expensive and may not provide a reliable result due to sampling error.10 Non-invasive methods to assess fibrosis can thus be an important tool to identify patients at risk for advanced fibrosis and mortality.10–14

What is already known about this subject?

► Non-alcoholic fatty liver disease (NAFLD) is associated with an increased risk of mortality.
► The ‘gold standard’ for staging hepatic fibrosis is a liver biopsy.
► NAFLD Fibrosis Scale (NFS) is a validated method for estimating fibrosis in NAFLD.

What are the new findings?

► Steatofibrosis, diagnosed with ultrasound and NFS is an independent predictor of overall and cardiac mortality.
► Using the NFS cut-off of 0.8 has the best predictive value for mortality.

How might it impact on clinical practice in the foreseeable future?

► This is an easy and non-invasive method to risk stratify subjects with NAFLD.
risk and is not easily accepted by patients.20 21 Among the
mated histologically through liver biopsy, which carries a
standard for establishing the stage of hepatic fibrosis is esti-
ation, and a number of follow-ups. Complete description
NHANES III consisted of extensive household interviews,
participants were enrolled and screened between the years of
Control and Prevention (CDC). For this survey, partici-
pants were enrolled and screened between the years of
The National Health and Nutrition Examination Survey
Methods
The National Health and Nutrition Examination Survey
 used for this study was conducted and
published by the National Center for Health Statistics
(NCHS), a subsidiary of the US Centers for Disease
Control and Prevention (CDC). For this survey, partici-
pants were enrolled and screened between the years of
1988 and 1994 at different locations across the USA. The
NHANES III consisted of extensive household interviews,
physical and dental evaluations, blood and urine collec-
tion, and a number of follow-ups. Complete description
of NHANES III selection and recruitment process is avail-
able from the CDC website.28
From the laboratory, interview and examination files,
the following parameters were extracted and used for this
study: age, gender, self-reported history of cardiovascular
diseases, history of smoking and alcohol consumption,
self-reported history and medication use for diabetes,
hypertension and hypercholesterolaemia, body mass
index (BMI), total blood cholesterol, high-density lipo-
protein (HDL), low-density lipoprotein (LDL), fasting
blood insulin and glucose, aspartate transaminase (AST),
alanine transaminase (ALT), serum albumin, platelet
count, viral hepatitis serology and transferrin saturation.
Diagnosis of NAFLD
Clinical, laboratory and US data were used to define
the diagnosis of NAFLD in this study.29 For NHANES III
participants, archived hepatobiliary US video images have
recently been re-reviewed by NCHS and published. In
this data collection, the presence of fat within the hepatic
parenchyma was graded as normal—mild or moderate—
severe. Quality control and quality assurance procedures
described elsewhere30 were used to standardise and val-
itate the readings of three US readers who had no access
to any other participants’ data.
For the purpose of ruling-out other causes of CLD, we
defined excessive alcohol consumption as >20 g/day for
men and ≥10 g/day for women in the year preceding
interview for NHANES III. Alcohol consumption was
evaluated using self-reported frequency of drinking (in
days of the year) and amount of drinking on a drinking
day. We also defined ‘suspected iron overload’ as trans-
ferrin saturation of ≥50%. Furthermore, individuals with
positive hepatitis B surface antigen (HBsAg) or hepa-
titis C virus antibody (anti-HCV) tests were presumed
to have viral hepatitis. Therefore, subjects used for this
study were presumed to have NAFLD if moderate-severe
hepatic steatosis was found by US in the absence of any
other possible cause of chronic liver disease listed above.
Furthermore, elevated liver enzymes were defined as ALT
≥40 IU/L in men, ≥31 IU/L in women, or AST ≥37
IU/L in men, ≥31 IU/L in women.

Staging of hepatic fibrosis by NFS
Using previously published formula,22 we calculated
NFS for all eligible participants with NAFLD diagnosed
by hepatic US. For this purpose, we used their age, BMI
measured at the time of examination, diabetes status
(present or absent, defined as fasting blood glucose
of ≥126 mg/dL or self-reported use of hypoglycaemic
agents), AST to ALT ratio, serum albumin and platelet
count. Individuals with any of these parameters missing
were excluded from the study. The NFS thresholds
recommended by the authors for ruling-in and ruling-out
hepatic fibrosis in NAFLD were applied.

Mortality follow-up
Mortality status for adult NHANES III participants was
reported as of 31 December 2011 by NCHS through the
US National Death Index (NDI), which is a computer-
ised database of all certified deaths in the USA since
1979. The NHANES III-Linked Mortality File publicly
available through NHANES website was used; overall and
cardiovascular mortality was collected. Follow-up length
measured in months was calculated as a period between
examination for NHANES III and death or the end of
follow-up, whichever was earlier. Individuals without
available mortality follow-up data were ineligible for the
study.

Statistical analysis
Of all NHANES III participants, only those with available
NFS value and mortality follow-up were included. Subjects
with high (above rule-in threshold), medium (between
the thresholds) and low (below rule-out threshold) NFS
values were compared using X² or Kruskall-Wallis test. In
this study, we did not apply sampling weights and stratified design as recommended by NHANES III, so no population-based conclusion could be made from our data. Rather, the described population was used only in a retrospective cross-sectional manner, and all reported associations are the associations observed in the studied cohort, which cannot be directly applied to the entire US population. Unless stated otherwise, p values of ≤0.05 were considered potentially significant.

Predictors of overall and cardiovascular mortality were evaluated using Cox proportional hazard model, with NFS or its binary transformations being used as potential predictors. Potential confounders used in the proportional hazard model for overall mortality were age, race, gender, obesity (BMI ≥30), diabetes status, self-reported history of cardiovascular disease, smoking, hypercholesterolaemia (total cholesterol>200 mg/dL, or LDL=139 mg/dL, or HDL <40 mg/dL for men or <50 mg/dL for women), hypertension (self-reported use of antihypertensive medication or blood pressure of ≥140/90 mm Hg) and elevated liver enzymes as determined at the time of examination. After bi-directional stepwise selection (significance level for entry 0.2, for stay −0.05), only predictors with a significant association with the outcome were left in the models. We also ran a round of sensitivity analysis by varying the NFS rule-in threshold to determine the value, which would return the best possible association with overall and cardiovascular mortality.

All analyses were run with SAS V.9.4. The study was granted an exemption from full review by Inova Institutional Review Board.

RESULTS

General characteristics of the study population

Of the total 20,050 adult participants from NHANES III, 2515 had complete data and fulfilled the diagnosis of NAFLD according to the definition described above. Of these NAFLD subjects, 129 had NFS >0.68 and comprised subjects with severe non-alcoholic steatofibrosis (referred as SF), whereas remaining 2386 NAFLD subjects had NFS <0.68 (no-SF). Comparison of demographic parameters of those with and without SF is shown in table 1. Expectedly, those with SF were older (63 vs 48 years), more likely to be white (45.7% vs 35.1%) and less likely to be men (33% vs 49%). Also, patients with SF demonstrated metabolically worse profile as compared with no-SF. The prevalence of obesity (77.5% vs 46.7%), diabetes (75.2% vs 17.6%), hypertension (65.1% vs 36.2%), metabolic syndrome (80% vs 55.5%), congestive heart failure (CHF) (14.2% vs 3.8%) and history of stroke (7% vs 2.6%) were significantly higher in the SF group (table 1).

Mortality data

After an average follow-up of 208 months, there were 754 deaths (table 1). At the end of follow-up period, the mortality rate in the SF group was significantly higher than the no-SF group (67.4% vs 28%, p<0.0001). In both groups, cardiovascular diseases and cancer were the leading causes of mortality.

Multivariate analysis

The association of NFS and mortality was evaluated in a series of multivariate survival analyses for overall mortality and for cardiovascular mortality. The analyses were categorised using the NFS as a continuous variable, a high NFS and low NFS, and a new threshold NFS of 0.8 for both overall and cardiac mortality after adjusting for several demographic and clinical variables.

Overall mortality

At first, the NFS was used as a continuous variable and tested as a predictor of overall mortality. After adjustment, the association of NFS with overall mortality was not statistically significant (aHR:1.06; 95% CI 0.97 to 1.13) (table 2). In an additional series of survival analyses with adjustment, different thresholds were selected for NFS and evaluated the association of the resulting binary transformation of NFS with mortality. The possible NFS thresholds were categorised as ‘high’ and ‘low’. The results showed that the association of binary transformation of NFS with overall mortality was significant for the ‘high’ categorisation (aHR:1.37; 95% CI 1.07 to 1.75, p=0.011), while the ‘low’ categorisation was not (aHR:0.91; 95% CI 0.76 to 1.09) (table 2). However, the best possible association of NFS with overall mortality was at the level of 0.8 (aHR:1.41; 95% CI 1.08 to 1.83, p=0.01). Besides NFS, other predictors of overall mortality were: age (aHR:1.07; 95% CI 1.07 to 1.08, p<0.0001), male gender (aHR:1.44; 95% CI 1.24 to 1.66, p<0.0001), black race (aHR:1.25; 95% CI 1.05 to 1.49, p=0.001), presence of hypertension (aHR:1.41; 95% CI 1.20 to 1.64, p<0.0001), diabetes (aHR:1.75; 95% CI 1.48 to 2.04, p<0.0001), CHF (aHR:1.78; 95% CI 1.39 to 2.28, p<0.0001), history of stroke (aHR:1.87; 95% CI 1.39 to 2.50, p<0.0001) and smoking (aHR:1.74; 95% CI 1.46 to 2.06, p<0.0001).

DISCUSSION

NAFLD is one of the most common causes of chronic liver disease, worldwide. The exact pathogenetic mechanism leading to mortality among patients with NAFLD has not yet been established. However, drastic changes in hormonal activity combined with dysregulation of cytokine production in individuals with advanced NASH have been recognised as possible contributors to negative outcomes. NAFLD has generally been categorised into simple steatosis and NASH, with subjects whose liver biopsies show evidence of NASH are considered to be at risk for progressive liver disease. In this context, stage of hepatic fibrosis in patients with NAFLD seems to be the sole consistent predictor of mortality, Therefore, the presence of steatofibrosis in NAFLD may be a more clinically relevant categorisation to determine the risk for overall and liver-specific mortality among patients with NAFLD. Nevertheless, in these studies, SF was determined histologically which relies on a liver biopsy with its
short comings. A non-invasive algorithm that can estimate SF will be clinically useful.

In this study, we show that steatofibrosis, diagnosed with a combination of hepatic ultrasonography and NFS, is an independent predictor of overall mortality. In fact, SF can also predict the most common cause of mortality in subjects with NAFLD, that is, cardiac mortality. Moreover, using a higher NFS cut-off as 0.8 can provide the best possible predictive value for mortality.

Table 1  The distribution of NFSs in NHANES III participants with NAFLD

| Variables                | High NFS* | Low or Med NFS† | Probability | Overall NAFLD cohort |
|--------------------------|-----------|-----------------|-------------|----------------------|
| **Demographic data**     |           |                 |             |                      |
| N                        | 129       | 2386            | 0.0000      | 2515                 |
| Age, years               | 62.78±11.09 | 47.96±15.33   | 0.0142      | 48.72±15.48          |
| Race                     |           |                 |             |                      |
| White                    | 59 (45.7%) | 838 (35.1%)    | 0.0154      | 897 (35.7%)          |
| Black                    | 39 (30.2%) | 506 (21.2%)    | 0.0000      | 545 (21.7%)          |
| Hispanic                 | 26 (20.2%) | 951 (39.9%)    | 0.0000      | 977 (38.8%)          |
| Male                     | 42 (32.6%) | 1166 (48.9%)   | 0.0003      | 1208 (48.0%)         |
| **Clinical data**        |           |                 |             |                      |
| BMI                      | 37.304±9.395 | 30.214±6.274  | 0.0000      | 30.577±6.654         |
| Waist circumference, cm  | 116.884±16.416 | 101.322±14.853 | 0.0000      | 102.097±15.310       |
| Obesity                  | 100 (77.5%) | 1115 (46.7%)   | 0.0000      | 1215 (48.3%)         |
| Glucose, mg/dL           | 165.429±91.884 | 112.727±51.749 | 0.0000      | 115.434±55.734       |
| Insulin                  | 27.552±21.139 | 18.164±19.834 | 0.0000      | 18.649±20.007        |
| Homeostasis Model        | 12.337±14.440 | 5.670±11.023   | 0.0000      | 6.015±11.318         |
| Assessment               |           |                 |             |                      |
| Total cholesterol, mg/dL | 214.674±49.724 | 212.407±45.646 | 0.8328      | 212.523±45.856       |
| HDL, mg/dL               | 47.685±20.348 | 45.458±13.973  | 0.5940      | 45.571±14.369        |
| LDL, mg/dL               | 120.212±33.238 | 130.079±37.888 | 0.1113      | 129.564±37.708       |
| Hypercholesterolaemia     | 108 (83.7%) | 1968 (82.5%)   | 0.7179      | 2076 (82.5%)         |
| Triglyceride, mg/dL      | 222.233±186.609 | 195.818±141.821 | 0.1597      | 197.173±144.529      |
| AST, IU/L                | 29.512±31.733 | 24.363±15.737  | 0.6044      | 24.627±16.956        |
| ALT, IU/L                | 18.318±14.758 | 24.006±21.072  | 0.0000      | 23.714±20.830        |
| Elevated liver enzymes   | 27 (20.9%)  | 404 (16.9%)    | 0.2405      | 431 (17.1%)          |
| Serum albumin, g/dL      | 3.864±0.356  | 4.144±0.365    | 0.0000      | 4.129±0.370          |
| Platelet count, 10^9     | 203.589±55.424 | 283.519±71.018 | 0.0000      | 279.419±72.472       |
| Hypertension             | 84 (65.1%)  | 864 (36.2%)    | 0.0000      | 948 (37.7%)          |
| Diabetes                 | 97 (75.2%)  | 421 (17.6%)    | 0.0000      | 518 (20.6%)          |
| Metabolic syndrome       | 76 (60.0%)  | 1153 (46.7%)   | 0.0000      | 1229 (56.6%)         |
| CHF                      | 18 (14.2%)  | 90 (3.8%)      | 0.0000      | 108 (4.3%)           |
| Stroke                   | 9 (7.0%)    | 62 (2.6%)      | 0.0035      | 71 (2.8%)            |
| Smoking                  | 18 (14.0%)  | 512 (21.5%)    | 0.0416      | 530 (21.1%)          |
| Non-Alcoholic Fatty Liver| 1.536±0.836 | −2.079±1.399   | 0.0000      | −1.894±1.590         |
| Score                    |           |                 |             |                      |
| Months of follow-up      | 151.357±78.005 | 210.950±56.808 | 0.0000      | 207.891±59.535       |
| Die                      | 87 (67.4%)  | 667 (28.0%)    | 0.0000      | 754 (30.0%)          |

*High NFS defined as NFS >0.676.
†Low or medium NFS defined as NFS ≤0.676.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CHF, congestive heart failure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAFLD, non-alcoholic fatty liver disease; NFS, NAFLD Fibrosis Score; NHANES III, National Health and Nutrition Examination Survey.
Our study makes a potentially valuable contribution to the literature, demonstrating the association between steatohepatitis and mortality. In fact, we assessed the predictive value of steatohepatitis with different cut-off levels of NFS in order to optimise the predictive value of the algorithm. In this context, the threshold with the best possible association with mortality was slightly higher than the conventional cut-off level for NFS (0.8 vs. 0.676). These data confirm the validity of NFS as a valuable prognostic tool in NAFLD.

This study has several limitations that need to be considered. The first limitation is that subjects were excluded due to unavailable NFS data. In addition, excluded subjects were also older and had higher BMI, suggesting that the distribution of NFS values in our cohort may be slightly biased towards lower values accompanied by lower mortality. Furthermore, the entire NHANES sample is not representative of the US population due to an oversampling of Mexican-Americans, which could cause bias since patients of different ethnicities are known to have different progression rates in chronic liver diseases. Another limitation is the unavailability of clinical follow-up, which could be useful for understanding the natural history of NAFLD progression and also, due to the length of follow-up, for monitoring those who may have developed NAFLD between examination and the end of follow-up. Finally, the study did not evaluate the association of NFS with most common long-term outcome, which is liver-related mortality.

In conclusion, this study suggests that severe non-alcoholic SF is associated with increased overall mortality. These data provide an easy and non-invasive method to risk stratify subjects with NAFLD for close monitoring and potential treatment candidacy.

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