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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 20 050 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 2.261), 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 mortality. These data should help clinicians to easily risk-stratify patients with NAFLD for close monitoring and treatment considerations in clinical trial setting.

Summary box

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 NALFD.

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.

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, such as cirrhosis, liver failure or hepatocellular carcinoma.[7–10]

The linkage between NAFLD and increased mortality has been shown in numerous studies.[11–14] Patients with NAFLD carry an increased risk of overall mortality, with cardiovascular diseases being the leading aetiology.[12–17] Furthermore, liver-related mortality is also higher in patients with NASH.[11 15–17] In fact, hepatic fibrosis stage ≥2 has been shown to be independently associated with liver-related mortality.[18 19] In this context, the 'gold standard' for establishing the stage of hepatic fibrosis is estimated histologically through liver biopsy, which carries a risk and is not easily accepted by patients.[20 21] Among the non-invasive biomarkers, NAFLD Fibrosis Score (NFS) is a simple, relatively reliable and widely validated methods for detecting advanced fibrosis in NAFLD.[22–26] In this context, NFS could provide a good estimate for stage of fibrosis in NAFLD. Additionally, ultrasound (US) has excellent sensitivity for hepatic steatosis.[27] A combination of US and NFS can easily and non-invasively establish the presence of steatosis and fibrosis in a relatively accurate manner. Therefore, the aim of the current study is to assess the long-term mortality and its associated predictors in subjects with significant non-alcoholic steatofibrosis (SF) as established by US and the NFS. Furthermore, we aimed to determine the best NFS threshold associated with SF and mortality.

METHODS

The National Health and Nutrition Examination Survey (NHANES III) 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, participants 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 collection, and a number of follow-ups. Complete description of NHANES III selection and recruitment process is available 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 lipoprotein (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 elsewhere[30] were used to standardise and validate 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 transferrin saturation of ≥50%. Furthermore, individuals with positive hepatitis B surface antigen (HBsAg) or hepatitis 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 of ≥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 computerised 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 X^2 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:1.372; 95% CI 1.07 to 1.75, p=0.011), while the 'low' categorisation was not (aHR:0.917; 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:1.411; 95% CI 1.08 to 1.83, p=0.01). Beside 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.[4] 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.[31 32] 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 seem to be the sole consistent predictor of mortality.[20 33–36] 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.[37] Nevertheless, in these studies, SF was determined histologically which relies on a liver biopsy with its

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		2515
Age, years	62.78±11.09	47.96±15.33	0.0000	48.72±15.48
Race				
White	59 (45.7%)	838 (35.1%)	0.0142	897 (35.7%)
Black	39 (30.2%)	506 (21.2%)	0.0154	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 Assessment	12.337±14.440	5.670±11.023	0.0000	6.015±11.318
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, $\times 10^9$	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 (80.0%)	1153 (55.5%)	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 Score	1.536±0.836	−2.079±1.399	0.0000	−1.894±1.590
Mortality data				
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.

short comings.[20 33–36] A non-invasive algorithm that can estimate SF will be clinically useful.[38]

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 2 Independent predictors of overall mortality in NHANES III

Predictors	aHR	95% CI	P value
NFS as a continuous predictor			
NFS	1.062	0.997 to 1.131	0.0599
Age, years	1.076	1.067 to 1.084	<0.0001
Male	1.424	1.228 to 1.65	<0.0001
Black	1.236	1.035 to 1.476	0.0193
Hypertension	1.398	1.196 to 1.635	<0.0001
Diabetes	1.709	1.44 to 2.028	<0.0001
CHF	1.781	1.391 to 2.279	<0.0001
Stroke	1.845	1.374 to 2.478	<0.0001
Smoking	1.748	1.471 to 2.076	<0.0001
Conventional NFS thresholds for high and low			
High NFS	1.372	1.073 to 1.755	0.0118
Low NFS	0.917	0.768 to 1.096	0.3407
Age, years	1.077	1.069 to 1.085	<0.0001
Male	1.441	1.242 to 1.672	<0.0001
Black	1.242	1.042 to 1.481	0.0156
Hypertension	1.404	1.201 to 1.642	<0.0001
Diabetes	1.694	1.433 to 2.003	<0.0001
CHF	1.768	1.382 to 2.261	<0.0001
Stroke	1.845	1.376 to 2.475	<0.0001
Smoking	1.741	1.467 to 2.067	<0.0001
Best NFS threshold of 0.8			
NFS (0.8)	1.411	1.085 to 1.834	0.0102
Age, years	1.078	1.071 to 1.086	<0.0001
Male	1.438	1.241 to 1.667	<0.0001
Black	1.251	1.05 to 1.49	0.0121
Hypertension	1.406	1.202 to 1.644	<0.0001
Diabetes	1.745	1.487 to 2.047	<0.0001
CHF	1.783	1.394 to 2.282	<0.0001
Stroke	1.872	1.396 to 2.509	<0.0001
Smoking	1.738	1.465 to 2.064	<0.0001

Adjusted HRs were calculated using Cox proportional hazard model.
CHF, congestive heart failure; 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 the literature, demonstrating the associations between steatofibrosis and mortality. In fact, we assessed the predictive value of steatofibrosis 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.[39 40] Another limitation is the unavailability of serial clinical follow-ups, 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 might have developed NAFLD between examination and the end of follow-up. Finally, the study did not evaluate the association of NFS with its most connatural 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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