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Association Between Noninvasive Fibrosis Markers and Mortality Among Adults With Nonalcoholic Fatty Liver Disease in the United States

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The clinical and public health significance of nonalcoholic fatty liver disease (NAFLD) is not well established. We investigated the long-term effect of NAFLD on mortality. This analysis utilized the National Health and Nutrition Examination Survey conducted in 1988-1994 and subsequent follow-up data for mortality through December 31, 2006. NAFLD was defined by ultrasonographic detection of hepatic steatosis in the absence of other known liver diseases. The presence and severity of hepatic fibrosis in subjects with NAFLD was determined by the NAFLD fibrosis score (NFS), the aspartate aminotransferase to platelet ratio index (APRI), and FIB-4 score. Of 11,154 participants, 34.0% had NAFLD—the majority (71.7%) had NFS consistent with lack of significant fibrosis (NFS <−1.455), whereas 3.2% had a score indicative of advanced fibrosis (NFS >0.676). After a median follow-up of 14.5 years, NAFLD was not associated with higher mortality (age- and sex-adjusted hazard ratio [HR]: 1.05; 95% confidence interval [CI]: 0.93-1.19). In contrast, there was a progressive increase in mortality with advancing fibrosis scores. Compared to subjects without fibrosis, those with a high probability of advanced fibrosis had a 69% increase in mortality (for NFS: HR, 1.69, 95% CI: 1.09-2.63; for APRI: HR, 1.85, 95% CI: 1.02-3.37; for FIB-4: HR, 1.66, 95% CI: 0.98-2.82) after adjustment for other known predictors of mortality. These increases in mortality were almost entirely from cardiovascular causes (for NFS: HR, 3.46, 95% CI: 1.91-6.25; for APRI: HR, 2.53, 95% CI: 1.33-4.83; for FIB-4: HR, 2.68, 95% CI: 1.44-4.99). *Conclusions*: Ultrasonography-diagnosed NAFLD is not associated with increased mortality. However, advanced fibrosis, as determined by noninvasive fibrosis marker panels, is a significant predictor of mortality, mainly from cardiovascular causes, independent of other known factors. (HEPATOLOGY 2013;57:1357-1365)

In the past 25 years, the prevalence of obesity in the United States has more than doubled, a trend that continues today without signs of slowing down.[1,2] In parallel, nonalcoholic fatty liver disease (NAFLD) has been recognized as the most prevalent liver disease in the United States and in many parts of the world.[2,3] However, the natural history of NAFLD is incompletely understood and its clinical and public health significance remains a matter of debate.

NAFLD is a clinicopathological entity that encompasses simple steatosis without fibrosis, nonalcoholic steatohepatitis (NASH) with varying stages of fibrosis, and cirrhosis. Patients with simple steatosis are thought to have benign prognosis,[4] whereas those with NASH may develop progressive liver disease.[5-7] One of the

Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; BMI, body mass index; CDC, Centers for Disease Control and Prevention; CIs, confidence intervals; CLD, chronic liver disease; CVD, cardiovascular disease; HOMA-IR, homeostatic model assessment of IR; HR, hazard ratio; IR, insulin resistance; NAFLD, nonalcoholic fatty liver disease; HCC, hepatocellular carcinoma; ICD-9, International Classification of Diseases, 9th Revision; MRS, magnetic resonance spectroscopy; NASH, nonalcoholic steatohepatitis; NFS, NAFLD fibrosis score; NHANES, National Health and Nutrition Examination Survey; PLT, platelet; DBP, diastolic blood pressure; SBP, systolic blood pressure; SE, standard error; UCOD_113, Underlying Cause of Death Recode-113; USG, ultrasonography.

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challenges in studying NAFLD in large groups of individuals is that the strict, traditional definition of NAFLD and NASH requires a liver biopsy, which makes it difficult to implement a population-based study.[8] Furthermore, characteristic features of NASH, such as steatosis, inflammation, and ballooning of hepatocytes, may diminish as fibrosis advances.[9,10]

Although a consensus is lacking as to optimal surrogate indicators for NAFLD and NASH for large-scale, population-based, epidemiological studies, a number of noninvasive tools may be considered. First, for the diagnosis of steatosis, abdominal ultrasonography (USG) has been shown to have a sufficient degree of diagnostic accuracy.[11] Second, methods to noninvasively diagnose hepatic fibrosis have been developed; they include serum marker panels and mechanical measures of liver stiffness, both of which have been correlated with hepatic fibrosis. Of those, the NAFLD fibrosis score (NFS) and FIB-4 are scoring systems validated to identify or exclude advanced fibrosis in patients with a diagnosis of NAFLD.[12-14] In addition, the aspartate aminotransferase (AST) to platelet (PLT) ratio index (APRI), originally created for chronic hepatitis C, is another simple marker that has been used for patients with NAFLD.[15,16]

In this study, we took advantage of the National Health and Nutrition Examination Survey (NHANES) data to determine the mortality effect of NAFLD and advanced fibrosis in NAFLD. NAFLD is defined by the ultrasonographic appearance of the liver, whereas NFS, APRI, and FIB-4 score were used to detect NAFLD with a discernible degree of fibrosis. Thus, the aim of our study was to investigate the effect of NAFLD in general and that of NAFLD with fibrosis on overall and cause-specific mortality in the U.S. adult population.

Patients and Methods

Subjects and Study Design. This study represents an analysis of the third NHANES data (1988-1994, the National Center for Health Statistics, the Centers for Disease Control and Prevention [CDC]), including the follow-up mortality data (NHANES III-Linked Mortality Files). NHANES employs a stratified, multistage, clustered probability sampling design to reach a representative sample of the noninstitutionalized civilian population in the United States.

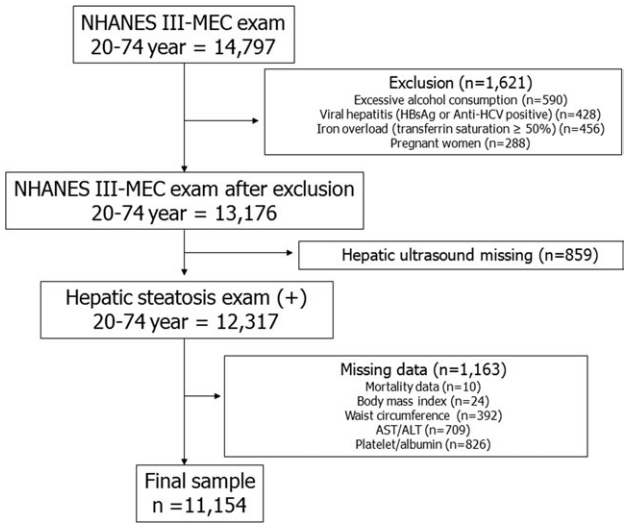

Fig. 1. Flow diagram of participants for the study.

Overall, 14,797 adult (20-74 years of age) participants of the NHANES III survey examined laboratory tests at a mobile examination center (Fig. 1). Of those, subjects with excessive alcohol consumption (>21 drinks/week in men and >14 drinks/week in women),[17] viral hepatitis (positive serum hepatitis B surface antigen and positive serum hepatitis C antibody), iron overload (transferrin saturation ≥50%), or pregnant women were excluded (n = 1,621). Of the remaining 13,176 participants, hepatic steatosis could be evaluated in 12,317 (93.5%). We removed subjects in whom data on serum aminotransferase, mortality status, or body mass index (BMI), waist circumference, albumin (ALB), or PLT count were missing. Thus, the final study sample consisted of 11,154 adults with complete data. The original survey was approved by the CDC's Institutional Review Board, and all participants provided written informed consent to participate. This analysis *per se* was deemed exempt by the institutional review board of the Mayo Foundation, because the data set used in the analysis was completely deidentified.

Clinical and Laboratory Evaluation. A wide array of demographic, lifestyle, and dietary information as well as anthropometric assessment and comprehensive

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laboratory data were available in the data set. Hypertension was defined as systolic blood pressure (SBP) $\geq$140 mmHg or diastolic blood pressure (DBP) $\geq$90 mmHg and/or previous use of antihypertensive medication. Diabetes mellitus was diagnosed in subjects with history of diabetes diagnosis and/or treatment with a hypoglycemic agent or insulin. Insulin resistance (IR) was defined by the top quartile of the homeostasis model assessment of IR (HOMA-IR; fasting glucose $\times$ fasting insulin/405) among subjects without diabetes in each gender. Current smokers were subjects who reported ongoing smoking or those who had smoked at least 100 cigarettes in the preceding 5 years. History of cardiovascular disease (CVD) was defined as self-reported history of congestive heart failure, stroke, or myocardial infarction.

NAFLD and Advanced Fibrosis. The original NHANES III examination included USG of the gallbladder at a mobile examination center as a part of the assessment for digestive diseases in adults 20-74 years of age. Subsequently, the archived gallbladder USG video images were reviewed to assess fatty liver.[18] Three USG reviewers were trained by a board-certified radiologist who specialized in hepatic imaging. Evaluation of fatty liver was performed using the following five criteria: (1) parenchymal brightness; (2) liver to kidney contrast; (3) deep beam attenuation; (4) bright vessel walls; and (5) gallbladder wall definition. Overall assessment, made using an algorithm based on these five criteria, reported normal versus mild, moderate, or severe hepatic steatosis.[18] For the purpose of this study, NAFLD was diagnosed in subjects with any degree (mild to severe) of steatosis.

In individuals with NAFLD, serum markers of fibrosis were used to assess severity of fibrosis. These included NFS, APRI, and FIB-4. NFS was calculated according to the published formula: NFS $= -1.675 + 0.037 \times$ age (years) $+ 0.094 \times$ BMI (kg/m^2) $+ 1.13 \times$ impaired fasting glycemia or diabetes (yes $= 1$, no $= 0$) $+ 0.99 \times$ AST/alanine aminotransferase (ALT) ratio $- 0.013 \times$ PLT ($\times 10^9$/L) $- 0.66 \times$ ALB g/dL).[12] Two cut-off points were selected to categorize subjects with NAFLD into three groups, including those with high probability (NFS $>$0.676), intermediate probability (NFS: 0.676~ -1.455), and low probability for advanced fibrosis (NFS < -1.455).[12] APRI was also calculated by the following published formula: APRI $=$ ([AST/upper limit of normal]/PLT count[10^9/L]) $\times$ 100.[15] We used the cut-offs for low and high probability of advanced fibrosis as published, namely, 0.5 and 1.5, respectively.[15] FIB-4 was calculated by the following formula: FIB-4 $=$ (age [years] $\times$ AST [U/L])/(PLT [10^9/L] $\times$ (ALT [U/L])$^{1/2}$). Published cut-off values were used to define low (FIB-4 $<$1.30), intermediate, and high (FIB-4 $>$2.67) probability of advanced fibrosis.[19]

Mortality. All participants of NHNAES III over 17 years of age were followed forward for mortality through December 31, 2006. The NHANES III–Linked Mortality File uses the Underlying Cause of Death Recode-113 (UCOD_113) code to classify all deaths according to the International Classification of Diseases, 9th Revision (ICD-9) for deaths before 1998 and to ICD-10 for those between 1999 and 2006.[20] Overall mortality and the following four items of cause-specific mortality were assessed: (1) cardiovascular (UCOD_113 55-63, 67-74), including ischemic heart disease, heart failure, atherosclerosis, CVD, aortic aneurysm, and other diseases of the arteries; (2) malignancy (UCOD_113 20-23, 25-36, 43), including all malignant neoplasms of solid organs; (3) liver related (UCOD_113 15, 24, 93-95), including chronic liver diseases (CLD), cirrhosis, and hepatocellular carcinoma (HCC); and (4) diabetic complications (E10-14).[20]

Statistical Analysis. The main tool for data analysis was the SAS callable SUDAAN 10.0.1 (Research Triangle Institute, Research Triangle Park, NC), which allows appropriate use of the stratified sampling scheme employed by NHANES to project the data to the U.S. population.[21]

We analyzed frequencies of categorical variables and means $\pm$ standard error (SE) of continuous variables (PROC CROSSTAB, PROC DESCRIPT). Baseline characteristics across groups were compared using the chi-square test for categorical variables and the two-sample *t* test or analysis of variance for continuous variables (PROC CROSSTAB, PROC REGRESS). Survival analysis, including overall and cause-specific mortality, utilized Cox's proportional hazards regression analysis (PROC SURVIVAL).

Results

NAFLD and Its Effect on Survival. The prevalence of NAFLD (mild to severe steatosis by USG) among the eligible subjects was 34.0%, which projected to a minimum of 43.2 million American adults. If the definition of NAFLD is restricted to moderate to severe steatosis, 20.2% were affected, corresponding to 25.6 million individuals. Demographic and clinical characteristics of subjects with NAFLD are summarized in Table 1 and are consistent with what is known of patients with NAFLD. For example, subjects with NAFLD were more likely to be older, male,

Table 1. Characteristics of Study Participants at Initial Survey*

Characteristics	NAFLD (n = 4,083)	No NAFLD (n = 7,071)	*P* Value
Age, years	45.5 ± 0.45	41.6 ± 0.44	<0.001
Gender, male, %	50.4 ± 1.23	45.8 ± 0.81	0.010
BMI kg/m^2	29.05 ± 0.23	25.47 ± 0.11	<0.001
Waist circumference, cm	98.35 ± 0.58	88.62 ± 0.27	<0.001
Race ethnicity, %			<0.001
Non-Hispanic white	75.2 ± 1.64	76.7 ± 1.32	
Non-Hispanic black	9.1 ± 0.70	11.0 ± 0.70	
Mexican American	6.7 ± 0.68	4.7 ± 0.42	
Smoking, %			<0.001
Never	45.1 ± 1.22	46.8 ± 1.21	
Ex-smoker	30.8 ± 1.37	23.6 ± 0.86	
Current smoking	24.1 ± 1.03	29.6 ± 1.06	
Alcohol consumption (drinks/week)	2.27 ± 0.12	2.54 ± 0.11	<0.001
Hypertension, %	31.0 ± 1.47	15.9 ± 0.63	<0.001
Diabetes, %	8.4 ± 0.53	2.6 ± 0.30	<0.001
History of CVD, %	6.4 ± 0.47	3.3 ± 0.36	<0.001
Lipid-lowering medication, %	4.3 ± 0.48	2.4 ± 0.30	<0.001
SBP, mmHg	124.6 ± 0.42	119.2 ± 0.34	<0.001
DBP, mmHg	76.4 ± 0.28	73.4 ± 0.22	<0.001
Total cholesterol, mg/dL	208.8 ± 1.46	201.2 ± 0.86	<0.001
HDL cholesterol, mg/dL	46.4 ± 0.44	51.9 ± 0.41	<0.001
Triglycerides, mg/dL	184.0 ± 3.66	121.8 ± 2.12	<0.001
ALT, IU/L	21.5 ± 0.59	15.4 ± 0.28	<0.001
AST, IU/L	22.9 ± 0.26	19.8 ± 0.17	<0.001
GGT, IU/L	34.8 ± 1.24	24.2 ± 0.41	<0.001
ALB, g/dL	4.18 ± 0.02	4.22 ± 0.02	<0.001
PLT, $\times 10^9$/L	273.7 ± 2.79	270.1 ± 2.09	<0.001
Glucose, mg/dL	104.9 ± 1.02	94.7 ± 0.49	<0.001
HbA1c, %	5.57 ± 0.03	5.25 ± 0.02	<0.001
Insulin, uU/mL (n = 9,798)	13.65 ± 0.37	8.41 ± 0.14	<0.001
HOMA-IR (n = 9,798)	3.67 ± 0.13	2.00 ± 0.04	<0.001

NAFLD was defined as ultrasonographic presence of mild to severe hepatic steatosis in the absence of any other possible cause of CLD. Insulin and HOMA-IR were also calculated from fasting subject only.

Abbreviations: HDL, high-density lipoprotein; GGT, gamma-glutamyltransferase; HbA1c, glycated hemoglobin.

*Mean ± SE.

hypertensive, and diabetic than those without steatosis. Similarly, BMI, waist circumference, plasma concentrations of total cholesterol and fasting glucose, and HOMA index were greater in NAFLD subjects.

Median follow-up in the 11,154 participants was 14.5 years (range, 0.03-18.1). There were a total of 1,795 deaths during the follow-up (15-year Kaplan-Meier survival: 83.7%). The most common cause of death was cardiovascular (9.3%) and malignancy (5.0%). Liver disease accounted for 0.4% of deaths. The 15-year unadjusted Kaplan-Meier survival in NAFLD subjects was 80.6%, compared to 85.5% in those without NAFLD. Table 2 summarizes results of Cox's regression analysis. After adjustment for age and sex, subjects with NAFLD had slightly and nonsignificantly higher overall mortality than those without NAFLD (hazard ratio [HR]: 1.05; 95% confidence interval [CI]: 0.93-1.19; $P = 0.431$). When additional demographic and clinical covariates, *such as race or* ethnicity, diabetes, and hypertension were taken into account, NAFLD had no association with mortality from all causes (HR, 0.89; 95% CI: 0.78-1.02). Similarly, NAFLD had no effect on cause-specific mortality. There were 37 deaths from liver-related causes, 19 of which occurred among NAFLD subjects. This gave rise to a fully adjusted HR for liver-related death of 1.90 with a wide CI, as expected from the small number of events. When the analysis was repeated with the definition of NAFLD restricted to moderate to severe steatosis, NAFLD had no demonstrable effect on mortality (data not shown).

NAFLD With Fibrosis and Survival. Of the subjects with NAFLD, 28.3% had NFS, consistent with an intermediate (25.1%) to high (3.2%) probability of fibrosis, whereas the remainder (71.7%) had a low probability. These data project to 10.8 million American adults with NAFLD and some evidence of advanced fibrosis, including 1.4 million with a high probability and another 9.4 million with an intermediate probability.

Table 3 compares those three groups of subjects with NAFLD using NFS. As expected from the component variables of the score, advanced fibrosis was associated with older age. There was a larger proportion of Non-Hispanic blacks and smaller portion of Mexican Americans among those with a high probability of advanced fibrosis. For most clinical parameters, increasing NFS was associated with more severe metabolic syndrome such as BMI, waist circumference, prevalence of hypertension and diabetes, and HOMA index. When APRI and FIB-4 were used for similar comparisons (data not shown), clinical and metabolic parameters of subjects with low to intermediate to high probabilities of advanced fibrosis were similar to the data presented in Table 3.

In Table 4, among NAFLD subjects, increasing NFS was associated with progressively higher risk of mortality—patients with a high probability of advanced fibrosis had a 69% increase in overall mortality (HR, 1.69; 95% CI: 1.09-2.63; after full adjustment), compared to the low probability group, whereas those with intermediate score had 26% increase in mortality (HR, 1.26; 95% CI: 0.98-1.64; after full adjustment). In cause-specific mortality analyses, the increase in mortality associated with fibrosis was essentially driven by cardiovascular causes. For example, subjects with a high NFS had 3.46-fold (95% CI: 1.91-6.25; after full adjustment) increase in cardiovascular mortality, compared to those with low

Table 2. Association Between NAFLD and Overall and Cause-Specific Mortality

		Age and Sex Adjusted		Multivariable Adjusted*	
	No. of Deaths	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Mortality from all causes	1,795				
No NAFLD (n = 7,012)	1,016	Reference		Reference	
NAFLD (n = 4,081)	779	1.05 (0.93-1.19)	0.431	0.89 (0.78-1.02)	0.101
CVD	673				
No NAFLD	381	Reference		Reference	
NAFLD	292	1.02 (0.85-1.22)	0.854	0.75 (0.56-1.01)	0.056
Liver disease	37				
No NAFLD	18	Reference		Reference	
NAFLD	19	1.16 (0.38-3.58)	0.788	1.90 (0.57-6.35)	0.290
Malignancy	430				
No NAFLD	263	Reference		Reference	
NAFLD	167	0.93 (0.72-1.20)	0.568	0.90 (0.65-1.26)	0.542
Diabetes	258				
No NAFLD	121	Reference		Reference	
NAFLD	137	2.18 (1.49-3.19)	<0.001	0.87 (0.59-1.29)	0.488

NAFLD was defined as ultrasonographic presence of mild to severe hepatic steatosis in the absence of any other possible cause of CLD.

*Multivariable models adjusted for age, sex, race or ethnicity, education, income, diabetes, hypertension, history of CVD, lipid-lowering medication, smoking status, waist circumference, alcohol consumption, caffeine consumption, total cholesterol, high-density lipoprotein cholesterol, transferrin saturation, and C-reactive protein.

Table 3. Characteristics of Study Participants With NAFLD According to NFS*

Characteristics	Low NFS (n = 2,755)	Intermediate NFS (n = 1,151)	High NFS (n = 173)	*P* Value
Age, years	40.7 ± 0.47	57.2 ± 0.55	61.2 ± 1.31	<0.001
Gender, male	50.8 ± 1.58	50.2 ± 2.34	43.7 ± 6.15	0.504
BMI, kg/m^2	27.78 ± 0.23	31.69 ± 0.41	36.64 ± 0.87	<0.001
Waist circumference, cm	94.74 ± 0.57	106.35 ± 0.92	117.06 ± 1.97	<0.001
Race/ethnicity, %				<0.001
Non-Hispanic white	73.8 ± 2.00	79.4 ± 1.87	74.5 ± 4.25	
Non-Hispanic black	8.3 ± 0.69	10.3 ± 1.08	15.4 ± 2.39	
Mexican American	7.7 ± 0.85	4.3 ± 0.46	3.5 ± 0.68	
Smoking, %				<0.001
Never	47.1 ± 1.33	39.3 ± 2.44	44.6 ± 5.70	
Ex-smoker	26.1 ± 1.46	43.0 ± 2.47	43.0 ± 5.09	
Current smoking	26.8 ± 1.21	17.7 ± 1.78	12.4 ± 3.56	
Alcohol consumption (drinks/week)	2.48 ± 0.13	1.79 ± 0.17	1.42 ± 0.48	<0.001
Hypertension, %	22.6 ± 1.50	50.9 ± 2.43	64.7 ± 5.60	<0.001
Diabetes, %	2.77 ± 0.35	18.7 ± 1.73	55.0 ± 4.98	<0.001
History of CVD, %	3.1 ± 0.47	13.5 ± 1.60	25.0 ± 5.95	<0.001
Lipid-lowering medication, %	3.0 ± 0.57	7.9 ± 1.35	5.9 ± 2.48	0.006
SBP, mmHg	121.1 ± 0.49	132.9 ± 0.73	137.5 ± 1.90	<0.001
DBP, mmHg	76.1 ± 0.35	77.3 ± 0.44	76.2 ± 1.24	<0.001
Total cholesterol, mg/dL	206.9 ± 1.61	213.7 ± 2.08	212.9 ± 6.45	<0.001
HDL cholesterol, mg/dL	46.7 ± 0.54	45.6 ± 0.61	46.7 ± 2.44	<0.001
Triglycerides, mg/dL	177.2 ± 3.19	197.9 ± 10.80	227.5 ± 20.45	<0.001
ALT, IU/L	22.8 ± 0.75	18.4 ± 0.64	17.7 ± 1.43	<0.001
AST, IU/L	22.8 ± 0.37	22.5 ± 0.46	28.0 ± 1.92	<0.001
Total bilirubin, mg/dL	0.61 ± 0.01	0.58 ± 0.01	0.63 ± 0.04	<0.001
Creatinine, mg/dL	1.06 ± 0.01	1.11 ± 0.01	1.19 ± 0.03	<0.001
GGT, IU/L	33.8 ± 1.46	35.3 ± 1.77	53.0 ± 7.99	<0.001
ALB, g/dL	4.24 ± 0.02	4.05 ± 0.02	3.89 ± 0.04	<0.001
PLT, $\times 10^9$/L	291.0 ± 3.32	233.3 ± 2.54	201.4 ± 6.83	<0.001
Glucose, mg/dL	104.9 ± 1.02	99.0 ± 2.26	147.4 ± 6.45	<0.001
HbA1c, %	5.38 ± 0.03	5.96 ± 0.07	6.89 ± 0.22	<0.001
Insulin, uU/mL (n = 3,536)	12.36 ± 0.35	16.41 ± 0.68	21.24 ± 1.37	<0.001
HOMA-IR (n = 3,536)	3.12 ± 0.11	4.78 ± 0.25	7.50 ± 0.80	<0.001

NAFLD was defined as ultrasonographic presence of mild to severe hepatic steatosis in the absence of any other possible cause of CLD. Insulin and HOMA-IR were also calculated from fasting subject only.

Abbreviations: HDL, high-density lipoprotein; GGT, gamma-glutamyltransferase; HbA1c, glycated hemoglobin.

*Mean ± SE.

Table 4. Association Between NFS and Overall and Cause-Specific Mortality Among Subjects With NAFLD

		Age and Sex Adjusted		Multivariable Adjusted*	
	No. of Deaths	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Mortality from all causes	778				
Low NFS	290	Reference		Reference	
Intermediate NFS	389	1.30 (1.00-1.70)	0.051	1.26 (0.98-1.64)	0.076
High NFS	99	2.17 (1.40-3.36)	<0.001	1.69 (1.09-2.63)	0.020
CVD	291				
Low NFS	88	Reference		Reference	
Intermediate NFS	162	2.01 (1.34-3.00)	0.001	2.16 (1.41-3.29)	<0.001
High NFS	41	3.69 (2.06-6.61)	<0.001	3.46 (1.91-6.25)	<0.001
Liver disease	19				
Low NFS	9	Reference		Reference	
Intermediate NFS	9	0.41 (0.06-2.89)	0.365	0.49 (0.08-2.83)	0.415
High NFS	1	0.05 (0.00-0.72)	0.029	0.07 (0.00-1.25)	0.070
Malignancy	167				
Low NFS	69	Reference		Reference	
Intermediate NFS	80	1.14 (0.64-2.03)	0.643	1.02 (0.54-1.95)	0.940
High NFS	18	1.33 (0.59-2.95)	0.483	1.03 (0.38-2.77)	0.953
Diabetes	137				
Low NFS	35	Reference		Reference	
Intermediate NFS	78	1.60 (0.84-3.02)	0.147	1.21 (0.52-2.84)	0.652
High NFS	24	4.46 (2.00-9.97)	<0.001	1.65 (0.50-5.41)	0.402

NAFLD was defined as ultrasonographic presence of mild to severe hepatic steatosis in the absence of any other possible cause of CLD. Because of smaller numbers of deaths, education, income, and history of CVD, lipid-lowering medication and C-reactive protein were excluded from model for liver disease.

*Multivariable models adjusted for age, sex, race or ethnicity, education, income, diabetes, hypertension, history of CVD, lipid-lowering medication, smoking status, waist circumference, alcohol consumption, caffeine consumption, total cholesterol, high-density lipoprotein cholesterol, transferrin saturation, and C-reactive protein.

NFS. Again, the number of liver-related deaths (n = 19) was too small to discern any trends.

When the analysis was repeated using APRI as a marker of fibrosis, results were overall identical to those obtained using NFS. In Table 5, for overall mortality, APRI increased the risk of mortality significantly with a multivariable HR of 1.85 (95% CI: 1.02-3.37) for high probability of advanced fibrosis. Similarly, high APRI was associated with CVD (HR, 2.53; 95% CI: 1.33-4.83). These results were essentially the same, when FIB-4 was used (Table 5). We conducted an additional sensitivity analysis by including HOMA-IR in the model, which did not change the results (data not shown). In another sensitivity analysis, cases with moderate to severe steatosis were compared to those with mild or no steatosis, which did not alter the results (data not shown).

Discussion

The main findings in this large, prospective, nationally representative, population-based study are that: (1) NAFLD, as detected by USG, by itself did not increase the risk of mortality, whereas (2) NAFLD with evidence of advanced fibrosis, defined here by non-invasive marker panels, was associated with increase in mortality. Furthermore, the increase in mortality was mainly attributable to cardiovascular causes.

NAFLD is common—a recent systemic review estimated the prevalence of NAFLD to be 10%-35% and that of NASH between 3% and 5% in the general population.[22] Despite its prevalence, the natural history of NAFLD is yet to be fully defined. It is understood that among individuals with NAFLD, simple steatosis is a benign condition, whereas NASH can progress to fibrosis, cirrhosis, and HCC. However, the prevalence of NASH and the incidence of sequelae of CLD in individuals with NAFLD in the population at large are difficult to obtain. Traditional histologic definitions of NASH, such as hepatocellular necroinflammation and ballooning, are poorly suited for epidemiological studies,[23] because it would be impractical, if not unethical, to obtain liver biopsies in asymptomatic community residents for a research purpose.

Given these limitations, previous investigators have used serum ALT activities and/or radiographic means to define NAFLD and distinguish between simple steatosis and NASH with and without fibrosis. To date, three articles have been published in which serum ALT data were used as a surrogate indicator of NAFLD in the NHANES III and NHANES III–Linked Mortality Files.[24-26] Although they were slightly different from

Table 5. Association Between APRI and FIB-4 Score and Overall and Cause-Specific Mortality Among Subjects With NAFLD

	APRI*		FIB-4*	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Mortality from all causes				
Low score	Reference		Reference	
Intermediate score	1.32 (0.78-2.23)	0.294	1.46 (1.16-1.82)	0.002
High score	1.85 (1.02-3.37)	0.044	1.66 (0.98-2.82)	0.060
CVD				
Low score	Reference		Reference	
Intermediate score	0.97 (0.40-2.34)	0.937	1.75 (1.26-2.43)	0.001
High score	2.53 (1.33-4.83)	0.006	2.68 (1.44-4.99)	0.003
Liver disease				
Low score	Reference		Reference	
Intermediate score	6.08 (0.77-48.21)	0.086	0.68 (0.11-4.05)	0.667
High score	3.01 (0.20-45.62)	0.420	1.32 (0.12-14.80)	0.821
Malignancy				
Low score	Reference		Reference	
Intermediate score	2.33 (0.91-5.96)	0.076	0.89 (0.49-1.63)	0.705
High score	2.31 (0.35-15.10)	0.374	0.96 (0.19-4.82)	0.962
Diabetes				
Low score	Reference		Reference	
Intermediate score	0.41 (0.12-1.46)	0.166	0.98 (0.57-1.68)	0.945
High score	29.36 (10.05-85.74)	<0.001	2.89 (0.33-25.35)	0.330

Because of smaller numbers of deaths, education, income, and history of CVD, lipid-lowering medication and C-reactive protein were excluded from model for liver disease.

*Models adjusted for age, sex, race or ethnicity, education, income, diabetes, hypertension, history of CVD, lipid-lowering medication, smoking status, waist circumference, alcohol consumption, caffeine consumption, total cholesterol, high-density lipoprotein cholesterol, transferrin saturation, and C-reactive protein.

one another in the study design, disparate conclusions were reached. One study found that ALT elevation was associated with an 8-fold increase in liver-related mortality, but not with overall mortality,[24] whereas another reported that increase in mortality was restricted to certain age groups only.[25] Serum ALT is a suboptimal indicator for NAFLD because it is neither sensitive nor specific for NAFLD. For example, a well-publicized population-based study observed that as many as 79% of subjects with hepatic steatosis, determined by magnetic resonance spectroscopy (MRS), had serum ALT within normal limits.[27] Obviously, serum ALT is entirely nonspecific for NAFLD and the accuracy of ALT in the detection of NAFLD depends on the degree to which other etiologies of liver disease can be confidently excluded. Furthermore, because serum ALT often decreases as fibrosis progresses in NAFLD patients, an important subgroup of NAFLD patients, namely, those with advanced fibrosis, may be systemically under-represented if ALT alone is used for detection of NAFLD.

To the extent that abdominal USG is widely used in clinical practice, detection of steatosis is one of the most commonly encountered scenarios in which NAFLD is suspected and diagnosed. Although USG may not be as accurate as MRS, its utility in the diagnosis of hepatic steatosis is quite high, as shown in a recent systemic review.[11] The main limitation of USG in the evaluation of patients with NAFLD is that it is unable to distinguish between NAFLD with and without advanced fibrosis, unless there are gross morphological changes consistent with cirrhosis. Because the USG description of hepatic steatosis has been released for the NHANES participants, a recent analysis of the NHANES data attempted to better define NASH by the combination of USG and serum ALT activities.[28] Using those definitions, the investigators found that neither NAFLD nor NASH had any effect on subsequent mortality. The main limitation of the study was the use of serum ALT in defining NASH, which, as discussed above, is a suboptimal surrogate.

Using the same data set, but employing a more-specific diagnostic marker for fibrosis, namely, the NFS, APRI, and FIB-4, we came to a slightly different conclusion—that is, NAFLD associated with evidence of fibrosis has a significant effect on subsequent mortality. It is noteworthy that most of the increase in mortality was the result of cardiovascular causes, even when typical risk factors for atherosclerotic disease, such as hypertension, diabetes, tobacco smoking, history of CVD, and lipid disorders, were already taken into account. This observation is consistent with previous data that NAFLD is an independent predictor of cardiovascular morbidity.[29-31]

With regard to mortality from liver disease, the lack of significant association between NAFLD with or

without fibrosis and mortality in this study should not be construed as a proof that NAFLD does not lead to morbidity and mortality from CLD. Instead, we believe that it is likely a type II error that, despite the large sample size of the NHANES study, the number of deaths from liver disease in the data set was too low to draw a firm conclusion. In addition, in patients with NAFLD, CVD represents such a strong competing risk that the study of the effect of NAFLD on liver-related mortality may require a much larger sample and/or longer follow-up. In the meantime, it may be fair to point out that the absolute risk of liver mortality in subjects with NAFLD in the general population is quite small. This is in contrast to previous investigations, frequently conducted in NAFLD patients who underwent liver biopsies at specialty liver clinics, which showed increased mortality from liver disease.[5-7,32] The difference between those and population-based studies such as ours is probably attributable to selection bias entailed in referral patients. Based on our data, we believe that, although it is wise to follow NAFLD patients with advanced fibrosis from the liver standpoint, it may be more important to pay attention to their cardiovascular risk to improve their overall outcome.

We do acknowledge limitations of this study. With regard to the assessment for steatosis and fibrosis, neither USG nor the fibrosis markers used in the study is an ideal diagnostic modality in an individual patient. For population-based epidemiological studies like ours, a balance needs to be sought between the accuracy of the diagnostic tools and feasibility of obtaining the diagnostic information. With regard to NFS, although it was originally developed in narrowly defined patient populations, it has subsequently been validated in heterogeneous groups of NAFLD patients as a correlate of liver histology, as shown in a recent meta-analysis, which incorporated 13 studies covering 3,064 patients of different ethnicities, ages, obesity, and diabetes status.[12,33-35]

Although its use is advocated by the practice guidelines,[17] for the purpose of this study, NFS has the limitation of including variables such as age and diabetes, which, in and of themselves, correlate with survival. Thus, a potential criticism is that the association between high NFS and mortality is confounded by those variables and not necessarily indicative of the effect of fibrosis. This consideration highlights the necessity and importance of multivariable analyses that incorporate appropriate adjustment for those and other relevant variables. In addition, replication of the same results in analyses based on APRI and FIB-4 adds to the confidence that the results are reproducible. Another potential concern for our data is the relatively large proportion (15.3%) of attrition of study subjects from the eligible NHANES III sample to the final analysis data set. A large part of this reduction was the result of lack of USG data and missing data of important variables. Availability of USG data has been reported to be random, and comparisons between the larger NHANES sample and that with complete data showed similar demographic characteristics.[36,37]

With these caveats in mind, we offer the following conclusions. First, as previously reported, NAFLD is highly prevalent among U.S. adults. Clearly, the prevalence of NAFLD is extremely high, which translates to a large aggregate disease burden, be it cardiovascular, diabetes, or liver related. Second, from this and other studies, it is clear that NAFLD without advanced fibrosis has little effect on mortality upon follow-up for up to two decades.[4,6,7,38] However, NAFLD with advanced fibrosis is an independent predictor of increased mortality, mainly from cardiovascular causes. In those patients, rigorous interventions to modify cardiovascular risk factors as well as careful follow-up for progression of fibrosis may be warranted.

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