Development and Validation of a Score for Fibrotic Nonalcoholic Steatohepatitis

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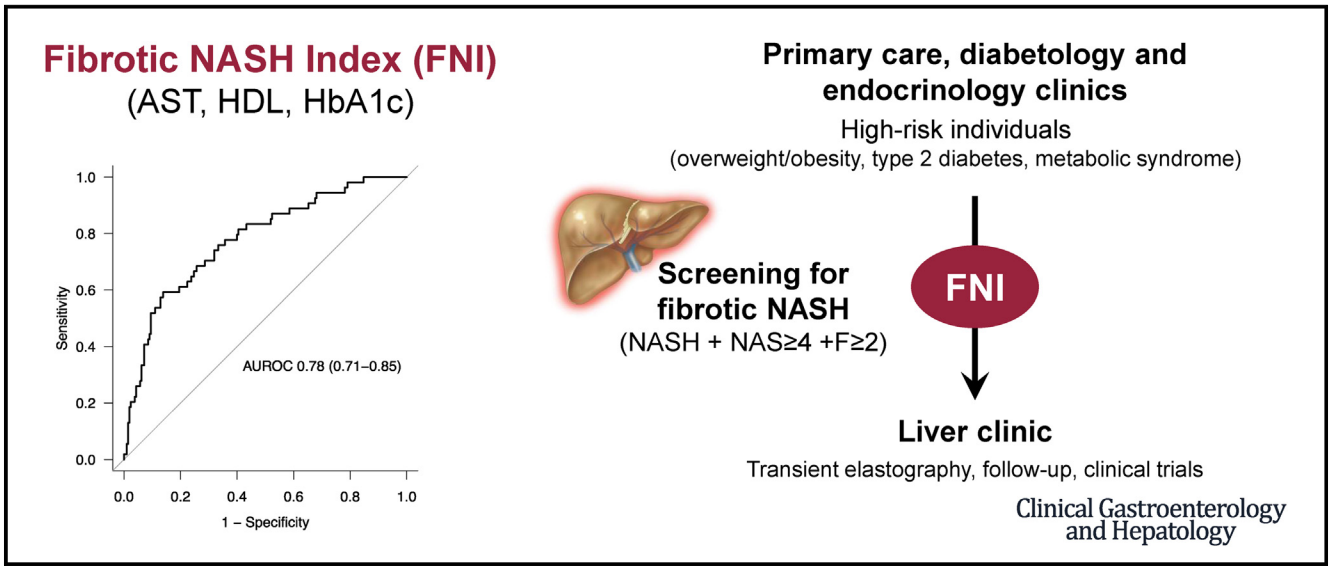

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Abbreviations used in this paper: AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic curve; BMI, body mass index; CAP, controlled attenuation parameter; CI, confidence interval; cT1, iron-corrected T1; F, fibrosis stage; FAST, FibroScan-AST; FIB-4, fibrosis-4 index; FNI, fibrotic NASH index; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; MRI, magnetic resonance imaging; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; NPV, negative predictive value; PDFF, proton density fat fraction; PPV, positive predictive value; SLD, severe liver disease; UK, United Kingdom.

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BACKGROUND & AIMS: **Noninvasive assessment of histological features of nonalcoholic fatty liver disease (NAFLD) has been an intensive research area over the last decade. Herein, we aimed to develop a simple noninvasive score using routine laboratory tests to identify, among individuals at high risk for NAFLD, those with fibrotic nonalcoholic steatohepatitis (NASH) defined as NASH, NAFLD activity score ≥4, and fibrosis stage ≥2.**

METHODS: **The derivation cohort included 264 morbidly obese individuals undergoing intraoperative liver biopsy in Rome, Italy. The best predictive model was developed and internally validated using a bootstrapping stepwise logistic regression analysis (2000 bootstrap samples). Performance was estimated by the area under the receiver operating characteristic curve (AUROC). External validation was assessed in 3 independent European cohorts (Finland, n = 370; Italy, n = 947; England, n = 5368) of individuals at high risk for NAFLD.**

RESULTS: **The final predictive model, designated as Fibrotic NASH Index (FNI), combined aspartate aminotransferase, high-density lipoprotein cholesterol, and hemoglobin A1c. The performance of FNI for fibrotic NASH was satisfactory in both derivation and external validation cohorts (AUROC = 0.78 and AUROC = 0.80–0.95, respectively). In the derivation cohort, rule-out and rule-in cutoffs were 0.10 for sensitivity ≥0.89 (negative predictive value, 0.93) and 0.33 for specificity ≥0.90 (positive predictive value, 0.57), respectively. In the external validation cohorts, sensitivity ranged from 0.87 to 1 (negative predictive value, 0.99-1) and specificity from 0.73 to 0.94 (positive predictive value, 0.12-0.49) for rule-out and rule-in cutoff, respectively.**

CONCLUSION: **FNI is an accurate, simple, and affordable noninvasive score which can be used to screen for fibrotic NASH in individuals with dysmetabolism in primary health care.**

Keywords: MAFLD; Metabolic Dysfunction-associated Fatty Liver Disease; Noninvasive tests; Obesity; Type 2 Diabetes.

Following the global burden of obesity and type 2 diabetes, nonalcoholic fatty liver disease (NAFLD) is now the major cause of chronic liver disease worldwide.[1] NAFLD encompasses a broad spectrum of conditions, from isolated hepatic fat accumulation to hepatocellular damage and inflammation (nonalcoholic steatohepatitis [NASH]), leading to fibrosis and end-stage liver disease, namely cirrhosis and hepatocellular carcinoma.[2,3] Obesity and type 2 diabetes are the strongest environmental factors increasing the risk of NAFLD.[4] However, despite the very large number of individuals with NAFLD, only a minority progress to cirrhosis and hepatocellular carcinoma.[1]

A body of evidence shows that individuals with fibrotic NASH, the inflammatory form of NAFLD associated with significant activity and fibrosis, are at risk of developing advanced liver disease.[5] The gold standard for diagnosing NASH and liver fibrosis is still a histological assessment by liver biopsy, an invasive and costly procedure that is not devoid of complications.[6,7]

The identification of individuals with fibrotic NASH in primary health care is crucial because these individuals will benefit the most from a referral to liver clinic for further investigation and follow-up. Moreover, these individuals are the ideal candidates for inclusion in NASH clinical trials.[8,9]

Therefore, due to the large number of individuals with NAFLD and the invasiveness of liver biopsy, noninvasive screening scores for fibrotic NASH are urgently needed. Indeed, existing scores are mainly focused on the assessment of liver fibrosis, the most relevant prognostic factor in NAFLD.[10,11] To date, 3 noninvasive scores have been specifically generated to assess fibrotic NASH, namely MACK-3,[12] NIS4,[13] and FibroScan-aspartate aminotransferase (FAST) score.[14] However, these scores are based on blood tests available only in highly specialized liver clinics or require instrumental evaluation by vibration-controlled transient elastography.

In this study, we aimed to develop a simple noninvasive score based on routine laboratory tests to screen for and identify fibrotic NASH in individuals at high risk for NAFLD in primary health care.

Methods

Derivation Cohort

MAFALDA cohort. A total of 264 participants from the "Molecular Architecture of FAtty Liver Disease in individuals with obesity undergoing bAriatric surgery (MAFALDA)" were included in the analyses.[15] Briefly, consecutive individuals with morbid obesity eligible for bariatric surgery, without history of alcohol abuse (≥30/20 g/day in men/women), chronic viral hepatitis, and other causes of liver disease, were recruited from May 2020 to June 2021 at Campus Bio-Medico University Hospital, Rome, Italy. Preoperative clinical and laboratory data were collected using standardized procedures.

Intraoperative liver biopsy was obtained and scored according to NAFLD activity score (NAS) classification.[16] NASH was diagnosed with at least grade 1 for steatosis, ballooning, and lobular inflammation.[17] Fibrotic NASH was defined as NASH, NAS $\geq$4, and fibrosis stage (F) $\geq$2. The MAFALDA study has been approved by the Local Research Ethics Committee (no. 16/20), and it was conducted in accordance with the principles of the Declaration of Helsinki. All participants gave written informed consent to the study.

External Validation Cohorts

Helsinki Cohort. A total of 328 consecutive individuals with morbid obesity eligible for bariatric surgery and 42 consecutive individuals with body mass index (BMI) $\geq$25 kg/m^2 undergoing liver biopsy for suspected NASH were recruited between 2006 and 2018 at Helsinki University Hospital, Helsinki, Finland. All participants were 18 to 75 years old, without history of alcohol abuse ($\geq$30/20 g/day in men/women), chronic viral hepatitis, and other causes of liver disease. A week before liver biopsy, participants underwent clinical examination and blood sampling as previously described.[18] Liver biopsies were scored according to NAS classification.[16] NASH was diagnosed when steatosis, lobular inflammation, and ballooning each had at least 1 grade.[19] Fibrotic NASH was defined as NASH, NAS $\geq$4, and F $\geq$2. The study was approved by the Local Research Ethics Committee at Helsinki University Hospital. All participants gave written informed consent to the study.

Liver Bible Cohort. A total of 947 consecutive individuals with dysmetabolism (at least 3 criteria among overweight [BMI >25 kg/m^2], hypertension [>130/85 mmHg or use of medication], hyperglycemia [>100 mg/dL], low high-density lipoprotein [HDL] cholesterol [<45/55 mg/dL in men/women], and increased triglycerides [>150 mg/dL]) were recruited from July 2019 to July 2021 at the Transfusion Center, Fondazione Ca' Granda Hospital, Milan, Italy.[20,21] All participants were 18 to 65 years old, without history of alcohol abuse ($\geq$30/20 g/day in men/women), chronic viral hepatitis, and other causes of liver disease, and were enrolled as part of a preventive medicine program among blood donors. Liver steatosis and fibrosis were noninvasively assessed by vibration-controlled transient elastography and controlled attenuation parameter (CAP) with FibroScan (Echosens, Paris, France), which was performed at the time of biochemical tests. Individuals at risk of fibrotic NASH were defined as those with FAST score >0.35.[14] The study was approved by the Local Research Ethics Committee at the Fondazione IRCCS Ca' Granda. All participants gave written informed consent to the study.

UK Biobank Cohort. The United Kingdom (UK) Biobank is a large prospective cohort study recruiting approximately 500,000 participants (age 40–69 years) between 2006 and 2010 throughout the UK.[22] The UK Biobank study has been approved by the North West Multicenter Research Ethics Committee (no. 11/NW/0274). All participants gave written informed consent to the study.

First, we selected unrelated UK Biobank participants of European ancestry based on our quality control pipeline, which has been described in detail previously.[20,23] Next, we included in our analyses only individuals with BMI $\geq$25 kg/m^2 and/or with type 2 diabetes as defined elsewhere.[24]

Then, to assess the performance of our score for fibrotic NASH, we selected 5368 individuals without chronic viral hepatitis and with liver magnetic resonance imaging (MRI) proton density fat fraction (PDFF) and iron-corrected T1 (cT1) measurements available.[25,26] Fibrotic NASH was defined as steatosis by PDFF >5.5%,[25] NASH by cT1 >800 msec,[27] and significant fibrosis by Fibrosis-4 (FIB-4) index $\geq$1.3.[28]

Finally, to assess the performance of our score for incident severe liver disease (SLD),[24] after excluding participants with MRI data available, we selected 305,745 individuals without liver disease at baseline and estimated those who developed SLD prospectively. Detailed information about the UK Biobank methods is provided in the Supplementary Appendix.

Statistical Analyses

The score was developed based on 264 morbidly obese individuals in the derivation cohort and internally validated using a bootstrapping stepwise logistic regression model (2000 bootstrap samples). A total of 15 predictors were included in the model: age, gender, BMI, waist circumference, glucose, hemoglobin A1c (HbA1c),

What You Need to Know

Background

Given the large and increasing number of individuals with nonalcoholic fatty liver disease, accurate and cost-effective noninvasive risk stratification tools for identifying fibrotic nonalcoholic steatohepatitis (NASH) are urgently needed.

Findings

Herein, we developed the Fibrotic NASH Index (FNI), an accurate, simple, and affordable noninvasive score for fibrotic NASH based on routine laboratory tests, namely aspartate aminotransferase, high-density lipoprotein cholesterol, and hemoglobin A1c.

Implications for patient care

FNI may be useful to rule out fibrotic NASH in at-risk individuals in primary health care and diabetology/endocrinology clinics. Additionally, FNI may help identify candidates for enrollment in NASH clinical trials, reducing screening failures.

total cholesterol, HDL cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase, gamma glutamyltransferase, platelet count, albumin, and total bilirubin. Logarithmic transformation was considered for continuous variables to improve the normality of distribution. Two (0.8%) individuals were removed from the analysis due to missing values. The score was derived based on the final predictors and the corresponding regression coefficients. Performance for fibrotic NASH was assessed by the area under the receiver operating characteristic curve (AUROC) in the derivation and validation cohorts. Rule-out and rule-in cutoffs were derived in the derivation cohort based on sensitivity $\geq$0.89 and specificity $\geq$0.90, respectively. Cutoff based on the maximal sum of sensitivity and specificity (Youden index) was also determined. At each cutoff, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were computed together with 95% confidence interval (CI). AUROCs were compared using the DeLong test. Calibration was assessed in the derivation cohort using Hosmer-Lemeshow goodness of fit test and calibration plot. Performance for incident SLD in the UK Biobank was estimated by AUROC of Cox proportional hazards models. Statistical analyses were performed using the software R, version 4.0.4 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Clinical Characteristics of Derivation and External Validation Cohorts

Clinical characteristics of derivation and external validation cohorts are shown in Table 1. The 2 histological cohorts (MAFALDA and Helsinki cohorts) were well-matched for age and gender, whereas the Liver Bible and UK Biobank cohorts had higher mean age and a higher rate of men. Biochemical parameters were similar across the cohorts. The Liver Bible cohort had the highest rate of hypertension (74% vs 41%–63%), whereas the Helsinki cohort had the highest rate of type 2 diabetes (38% vs 4%–16%). Biopsy-proven NASH was diagnosed in 42% individuals of the derivation cohort and in 12% individuals of the Helsinki cohort. Fibrotic NASH was reported in 20% individuals of the derivation cohort and in 2% to 5% of individuals of the external validation cohorts.

Development of a Prediction Model for Fibrotic NASH

Bootstrapping stepwise logistic regression analysis identified 3 final independent predictors of fibrotic NASH: AST, HDL cholesterol, and HbA1c. Based on the corresponding regression coefficients, the following index—the Fibrotic NASH Index (FNI)—was derived:

$$FNI = \frac{e^{(-10.33+2.54\times\ln AST\ [U/L]\ +3.86\times\ln \text{HbA1c}\ [\%]-1.66\times\ln \text{HDL}\ [mg/dL])}}{1+e^{(-10.33+2.54\times\ln AST\ [U/L]\ +3.86\times\ln \text{HbA1c}\ [\%]-1.66\times\ln \text{HDL}[mg/dL])}}$$

The FNI is a predicted probability score and ranges from 0 to 1. As an example, an individual with a FNI of 0.10 would have a 10% predicted probability of fibrotic NASH (NASH + NAS $\geq$4 + F $\geq$2). The FNI can be easily calculated on the following website: https://fniscore.github.io/.

In the derivation cohort, the performance of FNI for fibrotic NASH estimated by AUROC was 0.78 (95% CI, 0.71–0.85) with satisfactory calibration of predicted probabilities (Figure 1). In the external validation cohorts, AUROCs ranged from 0.80 to 0.95 (Table 2). In the derivation cohort, the cutoff for sensitivity $\geq$0.89 (rule-out zone) was 0.10, with a NPV of 0.93. The cutoff for specificity $\geq$0.90 (rule-in zone) was 0.33, with a PPV of 0.57 (Table 2). When applying these cutoffs to the external validation cohorts, at the rule-out cutoff of 0.10, sensitivity ranged from 0.87 to 1, with a NPV between 0.99 and 1; at the rule-in cutoff of 0.33, specificity ranged from 0.73 to 0.98, with a PPV between 0.12 and 0.49 (Table 2).

The performance of FNI and FIB-4 for fibrotic NASH was compared in derivation and 2 external validation cohorts (Figure 2, Table 2). Corresponding AUROCs were higher for FNI in the derivation and Liver Bible cohorts ($P = .001$ and $P = 3.08\text{x}10^{-08}$, respectively), whereas no difference was found between the 2 scores in the Helsinki cohort ($P = .85$).

Performance for Incident Severe Liver Disease

During a median follow-up of 9.0 years (interquartile range, 8.3–9.7 years), there were 1054 individuals who developed SLD, including 928 with cirrhosis and/or decompensated liver disease, 126 with hepatocellular carcinoma, and 18 that underwent liver transplantation. Death from SLD occurred in 542 individuals.

The AUROC of FNI for incident SLD was 0.77 (95% CI, 0.75–0.79), which was higher than the AUROC of FIB-4 (0.75; 95% CI, 0.73–0.77; $P = .03$) (Figure 3). At the FNI cutoff of 0.10 (rule-out zone), sensitivity was 0.81 vs 0.75 of FIB-4 cutoff of 1.3, with a NPV of 1 for both scores

Table 1. Clinical Characteristics of Derivation and External Validation Cohorts

	MAFALDA	Helsinki	Liver Bible	MRI UK Biobank
N	264	370	947	5368
Clinical data				
Age, *y*	43.4 (10.1)	49.1 (9.5)	53.9 (6.3)	55.3 (7.3)
Women	195 (74)	262 (71)	157 (17)	2406 (45)
BMI, kg/m^2	41.6 (4.4)	42.3 (7.7)	28.5 (3.1)	28.8 (3.4)
Metabolic profile				
Glucose, *mg/dL*	98 (92–106)	105 (96–114)	94 (87–103)	88 (83–95)
HbA1c, %	5.5 (5.3–5.9)	5.7 (5.4–6.2)	5.4 (5.2–5.6)	5.3 (5.1–5.6)
Cholesterol, *mg/dL*	179.1 (31.2)	163.8 (41.6)	202.1 (32.3)	224 (43)
HDL cholesterol, *mg/dL*	45.8 (9.8)	46.2 (12.1)	45.3 (10.1)	54 (12)
LDL cholesterol, *mg/dL*	121.3 (30.1)	99.1 (35.1)	123.3 (28.9)	143 (35)
Triglycerides, *mg/dL*	122 (90.8–164.2)	108 (80–145)	159 (114–199)	142 (106–204)
Liver function tests				
ALT, *U/L*	30.5 (20–41)	32 (22–46)	26 (21–35)	22.1 (16.7–30)
AST, *U/L*	26 (22–32)	29 (24–36)	23 (19–27)	24.8 (21.3–29.2)
GGT, *U/L*	25 (17.5–34)	31 (20–52)	23 (17–32)	28.2 (19.9–42.8)
Bilirubin, *mg/dL*	0.5 (0.4–0.7)	NA	NA	0.5 (0.4–0.6)
Albumin, *g/dL*	4.2 (0.3)	3.8 (0.4)	NA	4.5 (0.3)
Platelets, $10e^3/uL$	282.7 (63.4)	252.7 (63.0)	234.7 (51.5)	250.8 (56.6)
Comorbidities				
Hypertension	109 (41)	232 (63)	699 (74)	2236 (42)
Type 2 diabetes	41 (16)	141 (38)	35 (4)	405 (8)
Liver histology				
Steatosis grade			NA	NA
0	88 (33)	135 (37)		
1	93 (35)	153 (41)		
2	48 (18)	51 (14)		
3	35 (13)	31 (8)		
Lobular inflammation grade				
0	108 (41)	312 (84)		
1	143 (54)	48 (13)		
2	13 (5)	10 (3)		
3	0 (0)	0 (0)		
Ballooning grade				
0	22 (8)	318 (86)		
1	176 (67)	39 (11)		
2	66 (25)	13 (4)		
NASH	110 (42)	45 (12)		
NAS $\geq$4	109 (41)	42 (11)		
NASH + NAS $\geq$4 +F $\geq$2	54 (20)	17 (5)		
Fibrosis staging				
0	80 (30)	215 (58)		
1	117 (44)	121 (33)		
2	59 (22)	18 (5)		
3	7 (3)	10 (3)		
4	1 (0)	6 (2)		

Note: Continuous variables are shown as mean (SD) or median (IQR) as appropriate. Categorical variables are shown as number (percentage).
ALT, Alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; F, fibrosis stage; GGT, gamma glutamyltransferase; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MRI, magnetic resonance imaging; NA, not available; NAS, NAFLD Activity Score; NASH, non-alcoholic steatohepatitis; UK, United Kingdom.

(Table 3). A FNI >0.10 conferred a nearly 4-fold increased risk of incident SLD (adjusted hazard ratio, 3.55; 95% CI, 2.96–4.25; $P < .001$), which was higher than the increase in risk conferred by a FIB-4 $\geq$1.3 (adjusted hazard ratio, 3.0; 95% CI, 2.54–3.54; $P < .001$) (Table 3).

Discussion

In this study, we develop and validate the FNI, a novel and simple noninvasive score for detecting fibrotic NASH among individuals at high risk for NAFLD, namely those with overweight/obesity, type 2

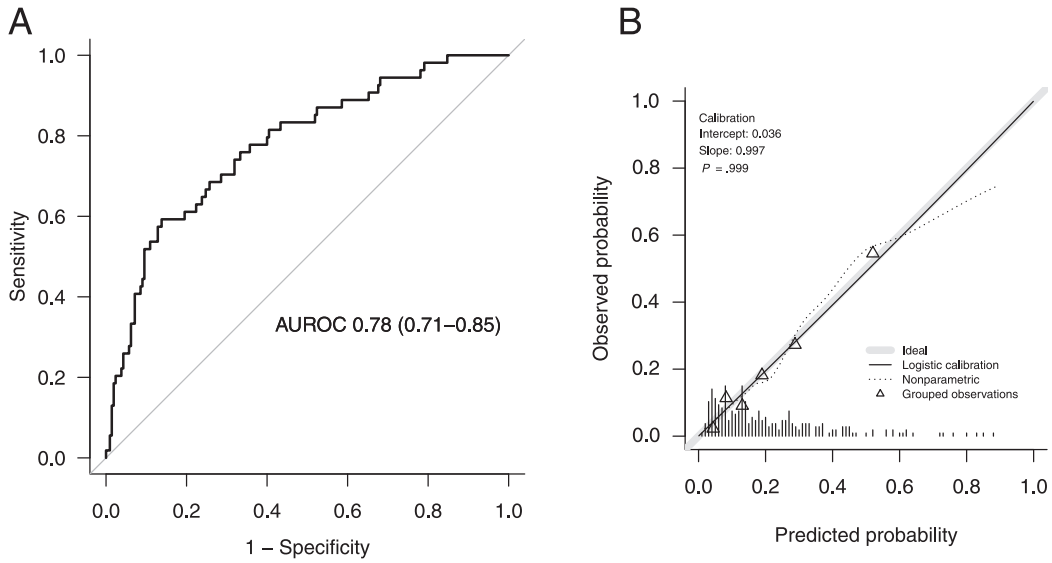

Figure 1. Diagnostic performance of FNI for fibrotic NASH in the MAFALDA cohort (n = 264). (*A*) ROC curve. Numbers in brackets are 95% CI. (*B*) Calibration plot. The *solid line* represents the ideal calibration. The *dashed line* represents the calibration estimated using locally estimated scatterplot smoothing (Loess). The *shaded area* indicates 95% CI. *Triangles* represent sextiles of participants grouped by similar predicted risk. *P* value is calculated using Hosmer-Lemeshow goodness of fit test.

diabetes, and metabolic syndrome. Notably, this is the first score tailored for fibrotic NASH based on routine laboratory tests, namely AST, HDL cholesterol, and HbA1c.

We started by examining the MAFALDA, a cross-sectional cohort of morbidly obese individuals in whom the diagnosis of fibrotic NASH was assessed by histology. In MAFALDA, we generated and internally validated a prediction model for fibrotic NASH by using a bootstrapping stepwise regression analysis. We found that AST, HDL cholesterol, and HbA1c were the best independent predictors of this condition. Consistently, elevated AST is a well-known biomarker of liver fibrosis,[29] whereas HbA1c and HDL cholesterol are both flagging the presence of dysmetabolism, given their correlation with insulin resistance and impaired glucose tolerance.[30,31] In the derivation cohort, this model showed good success in predicting fibrotic NASH with an AUROC of 0.78 (95% CI, 0.71–0.85).

Next, we validated our prediction model in 3 independent external cohorts comprising individuals with overweight/obesity, type 2 diabetes, and metabolic syndrome. In these cohorts, irrespective of the methodology used to assess fibrotic NASH (liver biopsy, vibration-controlled transient elastography including CAP, or liver MRI), the performance of our score was very good, with an AUROC range of 0.80 to 0.95. Notably, one of the external validation cohorts included more than 5000 high-risk individuals from the UK Biobank.

Existing noninvasive clinical scores are focused on detecting advanced fibrosis, the most relevant predictor of mortality in NAFLD.[10] However, the degree of liver inflammation is a crucial driver of liver damage.[32] In this scenario, the presence of NASH with significant activity (NAS $\geq$4) has been identified as an essential condition for enrollment in NAFLD clinical trials.[9] This is mainly due to 2 reasons: (1) the histological response to drug therapy is higher in individuals with an active disease,[33] and (2) the inclusion of individuals with fibrotic NASH is more likely to ensure that the estimated number of clinical events will occur during the study observation period. Along this line, the presence of an active liver disease is expected to be included among the prescribing criteria of new emerging pharmacotherapies once they become available. Within this context, FNI may also be used as a longitudinal biomarker to noninvasively monitor the effectiveness of interventional strategies for NASH.

Very recently, 3 noninvasive scores have been generated to detect fibrotic NASH: 2 blood-based, MACK-3[12] (AST, glucose, insulin, cytokeratin 18) and NIS4[13] (miR-34a-5p, alpha-2 macroglobulin, YKL-40, HbA1c), and the transient elastography-based FAST score (AST, CAP, liver stiffness measurement).[14] The accuracy of these scores for fibrotic NASH was good and comparable to that of FNI, with AUROCs ranging from 0.80 to 0.85. However, these scores are based on blood/instrumental tests that are relatively expensive and/or not widely available in primary care. Consequently, although FibroScan is increasingly used worldwide, the screening for fibrotic NASH in large at-risk populations in primary care using these scores appears to be impractical and costly.

Would the FNI score be a viable option to screen for fibrotic NASH in large at-risk populations? Within this context, the risk stratification pathway recently proposed by the European Association for the Study of the Liver recommended a FIB-4 cutoff $<$1.3 to rule out those not

Table 2. Diagnostic Performance of FNI and FIB-4 for Fibrotic NASH and Cutoff Values in Derivation and External Validation Cohorts

	MAFALDA	Helsinki	Liver Bible	MRI UK Biobank
N	264	370	947	5368
Fibrotic NASH definition	NASH + NAS ≥4 + F ≥2	NASH + NAS ≥4 + F ≥2	FAST score >0.35	PDFF >5.5% + cT1 >800 msec + FIB-4 ≥1.3
Fibrotic NASH, n (%)	54 (20)	17 (5)	37 (4)	118 (2)
FNI AUROC (95% CI)	0.78 (0.71–0.85)	0.83 (0.72–0.95)	0.95 (0.92–0.98)	0.80 (0.75–0.83)
FIB-4 AUROC (95% CI)	0.63 (0.54–0.71)	0.82 (0.72–0.92)	0.68 (0.58–0.78)	NA
FNI ≥0.30 (Youden index)				
n (%)	59 (22.3)	124 (33.5)	52 (5.5)	433 (8.1)
Sensitivity	0.57	0.88	0.62	0.39
Specificity	0.87	0.69	0.97	0.93
PPV	0.53	0.12	0.44	0.11
NPV	0.89	0.99	0.98	0.99
FNI ≤0.10 (Rule-out zone)				
n (%)	83 (31.4)	77 (20.8)	464 (50)	2526 (47.1)
Sensitivity	0.89	0.94	1	0.87
Specificity	0.37	0.22	0.51	0.54
PPV	0.27	0.06	0.08	0.04
NPV	0.93	0.99	1	0.99
FNI ≥0.33 (Rule-in zone)				
n (%)	49 (18.6)	109 (29.4)	41 (4.3)	337 (6.3)
Sensitivity	0.52	0.82	0.54	0.34
Specificity	0.90	0.73	0.98	0.94
PPV	0.57	0.13	0.49	0.12
NPV	0.88	0.99	0.98	0.98
FIB-4 ≥1.3				
n (%)	21 (8.0)	111 (30.0)	216 (22.8)	NA
Sensitivity	0.11	0.76	0.53	NA
Specificity	0.93	0.72	0.78	NA
PPV	0.29	0.12	0.09	NA
NPV	0.80	0.98	0.98	NA

Note: Optimal cutoffs for fibrotic NASH were obtained in the derivation cohort based on the maximal sum of sensitivity and specificity (Youden index), on sensitivity ≥89% (rule-out zone), and on specificity ≥90% (rule-in zone).
AUROC, Area under the receiver operating characteristic curve; CI, confidence interval; cT1, iron-corrected T1; F, fibrosis stage; FAST, FibroScan-AST score; FIB-4, fibrosis-4 index; FNI, fibrotic NASH index; MRI, magnetic resonance imaging; NA, not applicable; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; NPV, negative predictive value; PDFF, proton density fat fraction; PPV, positive predictive value; UK, United Kingdom.

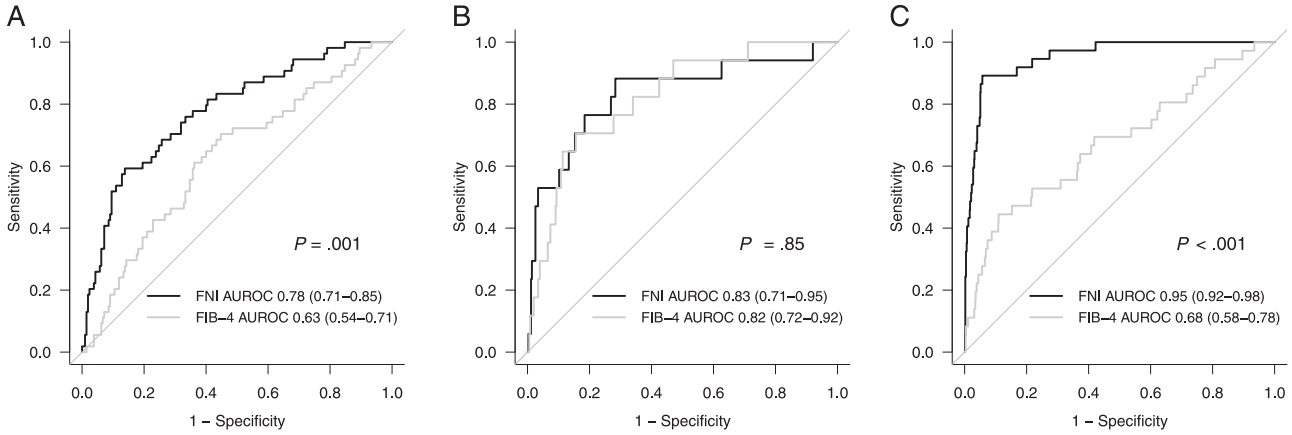

Figure 2. ROC curves for fibrotic NASH by FNI and FIB-4 in the (*A*) MAFALDA cohort (n = 264), (*B*) Helsinki cohort (n = 370), and (*C*) Liver Bible cohort (n = 947). Numbers in brackets are 95% CI. *P* values are calculated using the DeLong test. *P* values < .05 are considered statistically significant.

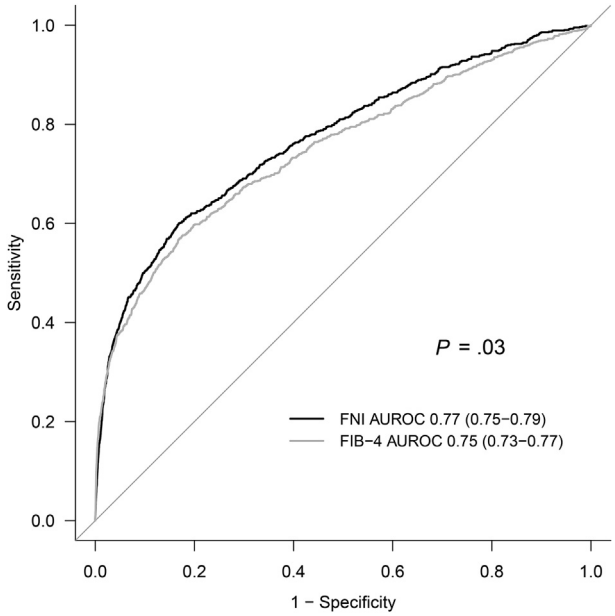

Figure 3. ROC curves for incident severe liver disease by FNI and FIB-4 in the UK Biobank (n = 305,745). Numbers in brackets are 95% CI. *P* values are calculated using the DeLong test. *P* values < .05 are considered statistically significant.

needing a referral to the liver specialist.[34] In individuals with metabolic risk factors from the general population, a FNI value ≤0.10 (rule-out zone) would exclude the presence of fibrotic NASH with high sensitivity and high NPV. Importantly, in both derivation and external validation cohorts, at least 1 of 5 individuals belonged to the rule-out zone, thus avoiding further referral to the liver specialist. Notably, the FNI cutoff of 0.10 had a higher sensitivity for fibrotic NASH as compared with the FIB-4 cutoff of 1.3. Consequently, in the general population with metabolic risk factors, the risk stratification using FNI as opposed to FIB-4 would allow to miss fewer individuals with fibrotic NASH. Importantly, these individuals may require and benefit the most from a prompt intervention in liver clinics due to the presence of an active disease at higher risk of liver-related outcomes. Consistently, we found that, during a median follow-up of 9 years, FNI was more accurate than FIB-4 for predicting incident SLD. However, it is fair to say that FIB-4 has been generated to assess liver fibrosis, and the 1.3 cutoff is used to rule out advanced fibrosis rather than fibrotic NASH.[34]

Table 3. Diagnostic Performance of FNI and FIB-4 for Incident Severe Liver Disease in the UK Biobank (n = 305,745)

	FNI	FIB-4
AUROC (95% CI)	0.77 (0.75–0.79)	0.75 (0.73–0.77)
Cutoff	>0.10	≥1.3
n (%)	127,460 (51.1)	119,658 (43.0)
HR (95% CI)	4.21 (3.55–5.01)[a]	4.10 (3.54–4.75)[b]
aHR (95% CI)	3.55 (2.96-4.25)[a]	3.0 (2.54-3.54)[b]
Sensitivity	0.81	0.75
Specificity	0.49	0.57
PPV	0.01	0.01
NPV	1	1

Note: HRs with 95% CIs were calculated by Cox proportional hazards models.
Note: Age, gender, and alcohol intake (g/day) were included in the multivariable models.
aHR, Adjusted HR; AUROC, area under the receiver operating characteristic curve; CI, confidence interval; FIB-4, fibrosis-4 index; FNI, fibrotic NASH index; HR, hazard ratio; PPV, positive predictive value.
[a]*P* < .001 vs FNI ≤.10.
[b]*P* < .001 vs FIB-4 <1.3.

Conversely, PPV for fibrotic NASH was rather low in the FNI rule-in zone. This is mainly due to the low prevalence of fibrotic NASH in the cohorts used in our study. Indeed, the performance of any disease predictive model is highly dependent on the prevalence of the disease in the referral population.[34] Indeed, although FNI was generated and validated in individuals at high risk for NAFLD, the prevalence of fibrotic NASH in these individuals was relatively low. However, the performance of the FNI rule-in cutoff is expected to be higher in individuals from secondary/tertiary care centers where the prevalence of advanced fibrosis is higher. Further studies are warranted to assess the performance of FNI in these settings.

Collectively, our data support that FNI may be useful for ruling out rather than diagnosing fibrotic NASH in at-risk individuals in primary health care and diabetology/endocrinology clinics. Individuals with indeterminate and positive results would deserve referral to liver clinic for further investigations and follow-up.

The present study has several strengths. First, we used a large and well-characterized derivation cohort with liver biopsy data available. Second, we developed, for the first time, a predictive model for fibrotic NASH based on routine and widely available laboratory tests that are commonly evaluated in individuals with metabolic risk factors. Third, we validated our findings in 3 independent and large external validation cohorts. Among them, 1 included more than 5000 individuals from the UK Biobank.

Our study has also some limitations. First, FNI has been specifically designed and validated in individuals with dysmetabolism and not in those referred for NAFLD in liver secondary/tertiary care settings. Therefore, its performance should be further verified before being used in this context. Second, we could not compare FNI with other noninvasive blood-based scores for fibrotic NASH, such as MACK-3, because they were not available in most cohorts.

Conclusion

In conclusion, we developed and validated the FNI, an accurate, simple, and affordable noninvasive score for

fibrotic NASH based on routine laboratory tests, namely AST, HDL cholesterol, and HbA1c. This score may help clinicians identify at-risk individuals in primary health care and diabetology/endocrinology clinics who require a referral to the liver specialist.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at http://doi.org/10.1016/j.cgh.2022.03.044.

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Conflicts of interest
The authors disclose no conflicts.

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Supplementary Appendix

UK Biobank

The United Kingdom (UK) Biobank is a large prospective cohort study recruiting approximately 500,000 participants (age 40–69 years) between 2006 and 2010 from 22 assessment centers throughout the UK.[1] Clinical information and laboratory data were collected using highly standardized procedures. Medical diagnoses were obtained through linkage of hospital admissions, death register, and cancer register from the National Health Service records (data fields 41270, 40001, 40002, and 40006).

Magnetic Resonance Imaging (MRI) UK Biobank Cohort

To assess the performance of our score for fibrotic nonalcoholic steatohepatitis, we selected a total of 5368 European individuals with overweight/obesity and/or type 2 diabetes, without chronic viral hepatitis (International Classification of Diseases 10th edition [ICD-10] B18–B19) from hospital admissions and death register, and with liver magnetic resonance imaging proton density fat fraction, and iron-corrected T1 measurements available. Participants were scanned at the UK Biobank Imaging Centre in Cheadle (UK) using a Siemens 1.5T MAGNETOM Aera as described in detail elsewhere.[2,3] Briefly, a shortened modified look locker inversion was used to quantify liver T1 and a multi echo-spoiled gradient-echo was used to quantify liver iron and fat. Data were analyzed using LiverMultiScan Discover 4.0 software.

Prospective UK Biobank Cohort

To assess the performance of our score for incident severe liver disease (SLD), we selected a total of 305,745 European individuals with overweight/obesity and/or type 2 diabetes, after excluding those with proton density fat fraction and iron-corrected T1 measurements available. Baseline exclusion criteria were: (1) self-reported history or hospital diagnosis of chronic viral hepatitis, SLD, or other causes of liver disease (ICD10 B18, B19, C22.0, E83.0, E83.1, I85.0, I85.9, K70.3, K70.4, K70.9, K71, K72.1, K72.9, K74.1, K74.2, K74.3, K74.4, K74.5, K74.6, K75.2, K75.3, K75.4, K75.8, K75.9, K76.6, K76.7, K76.8, K76.9, R18, Z94.4); (2) self-reported history or diagnosis from cancer register of liver cancer (ICD-10 C22); (3) missing data for any score variable. SLD was defined as a composite diagnosis of cirrhosis, decompensated liver disease, hepatocellular carcinoma, and/or liver transplantation from hospital admissions, death register, and cancer register (ICD-10 C22.0, I85.0, I85.9, K70.3, K70.4, K72.1, K72.9, K74.1, K74.2, K74.6, K76.6, K76.7, Z94.4). Follow-up length was calculated from the date of baseline assessment visit up to the first date of SLD diagnosis, the date of death, or the date of end of follow-up for the assessment center attended (January 31, 2018), whichever occurred first. Participants were excluded from the analyses if they received hospital diagnosis of competing liver diseases (ICD-10 B18, B19, E83.0, E83.1, K71, K74.3, K74.4, K74.5, K75.2, K75.3, K75.4, K75.8, K75.9) before the diagnosis of SLD.

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