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ORIGINAL RESEARCH—CLINICAL

A Real-World Experience Utilizing the FAST Score to Identify Patients With Nonalcoholic Steatohepatitis Fibrosis

Gres Karim,[1] Dewan Giri,[1] Brooke Wyatt,[2] and Amreen M. Dinani[3,4]

[1]Department of Medicine, Mount Sinai Beth Israel Medical Center, Icahn School of Medicine at Mount Sinai, New York, New York; [2]Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, New York; [3]Division of Liver Diseases, Icahn School of Medicine at Mount Sinai, New York, New York; and [4]Department of Gastroenterology and Hepatology, Duke University, Durham, North Carolina

BACKGROUND AND AIMS: We aimed to test the performance of the Fibroscan-aspartate aminotransferase (FAST) score, a noninvasive test, to identify nonalcoholic steatohepatitis (NASH) and significant fibrosis (NASH + ≥F2) in a cohort of patients with a histological diagnosis of NASH, using a cutoff of ≥0.35 as a rule in factor. We also compared performance to liver stiffness measurement (LSM) ≥8 kPa and the fibrosis-4 index (FIB-4) ≥1.3 and attempted to identify risk factors to develop a model for improving diagnostic accuracy. **METHODS:** Patients with histologically confirmed NASH were identified from 2020–2021. Demographic information, laboratory data, and LSM were collected. The FAST score and FIB-4 were calculated. Univariate and backward entry multivariate logistic regression analyses were performed to identify risk factors in addition to the FAST score ≥0.35 that are associated with an accurate histological diagnosis of NASH + ≥F2. Discrimination and overall accuracy were assessed using area under receiver operating characteristic curves. **RESULTS:** Using a rule in cutoff of ≥0.35, the FAST score performed with a sensitivity, specificity, negative predictive value, and positive predictive value of 96.4%, 36.8%, 77.7%, and 81.8%, respectively. Age ($P = .05$) and FAST ≥0.35 ($P = .001$) correctly identified histologically confirmed NASH + ≥F2. The FAST + age model outperformed FAST ≥0.35 (0.70, confidence interval [CI]: 0.55–0.84), LSM ≥8 kPa (0.72, CI: 0.59–0.85), and FIB-4 ≥1.3 (0.73, CI: 0.59–0.87) with a c-statistic of 0.78 (CI: 0.64–0.92). **CONCLUSION:** A FAST score with a rule cutoff of ≥0.35 performed well (c-statistic: 0.70) and was superior to LSM and FIB-4 when age was incorporated into the model (0.78) in detecting NASH + ≥F2 fibrosis in the real world.

Keywords: Noninvasive Markers of Fibrosis; Nonalcoholic Fatty Liver Disease; Liver Stiffness Measurement; Fibrosis-4 Index

Introduction

The global impact of nonalcoholic fatty liver disease (NAFLD) is astonishing, with a prevalence of about 24%.[1] In the United States, NAFLD and nonalcoholic steatohepatitis (NASH) are recognized as one of the leading causes of chronic liver disease, estimated to affect 30% and 5% of the population, respectively.[2] NAFLD is currently the most common indication for liver transplantation in the United States.[3] NASH, the more aggressive form, has the potential to develop into advanced fibrosis, cirrhosis, and hepatocellular carcinoma if left untreated.[1,4] The economic impact of NAFLD is as staggering as its clinical consequences. A recent study utilized Markov-based decision-analytic models to estimate the clinical and economic burden of NAFLD in the United States and Europe. They ascertained that over 64 million people in the United States were projected to have NAFLD and estimated an annual direct medical cost of $103 billion ($1613 per patient). In Europe (represented by Germany, France, Italy, and the United Kingdom), about 52 million people are affected by NAFLD and contribute an estimated annual direct medical cost of €35 billion (from €354 to €1163 per patient).[5]

Making the diagnosis of NAFLD is complex, essentially a diagnosis of exclusion. The definitive diagnosis of NASH requires the histopathological presence of hepatic steatosis, hepatocellular injury (ballooning), and inflammation, as determined by a liver biopsy.[6,7] Accurate diagnosis of NASH and stage of fibrosis is one of the challenges in the NAFLD paradigm. While liver biopsy is the standard for diagnosis and is required for eligibility for pharmacology-related clinical trials and determination of endpoints, it is flawed and impractical. Though liver biopsy is arguably effective in diagnosis of the initial disease, interobserver variation exists. Liver biopsy is an invasive procedure associated with small but significant complications and remains a time-consuming method to guide treatment decisions, follow

Abbreviations used in this paper: **ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUROC, area under receiver operating characteristic; BMI, body-mass index; CAP, controlled attenuation parameter; CI, confidence interval; ≥F2, significant fibrosis; ≥F3, stage 3 fibrosis and greater; FAST, FibroScan-AST; FIB-4, fibrosis-4 index; LSM, liver stiffness measurement; NAFLD, nonalcoholic fatty liver disease; NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis; NASH CRN, nonalcoholic steatohepatitis clinical research network; NITs, noninvasive tests; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value; T2DM, type 2 diabetes mellitus; TG, triglycerides; VCTE, vibration-controlled transient elastography.**

Most current article

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disease progression, and monitor the effectiveness of treatment.[8]

The need to develop noninvasive and practical methods to diagnose NASH fibrosis is one of the tasks the NAFLD community recognizes as important and necessary. Several noninvasive tests (NITs) have been studied and validated with moderate predictive capability to accurately rule out advanced fibrosis.[8] These tests include serum-based markers, such as the NAFLD fibrosis score, fibrosis-4 index (FIB-4), and aspartate aminotransferase (AST)/platelet ratio index, and radiological-based markers, such as vibration-controlled transient elastography (VCTE) and magnetic resonance elastography.[8] These, in combination with high-risk clinical characteristics including obesity, type 2 diabetes mellitus (T2DM), and laboratory parameters (eg, aminotransferases), can aid in improving our diagnostic accuracy of NASH fibrosis. The FibroScan-AST (FAST) score is a NIT that uses a combination of serum and radiological parameters proposed to identify patients with NASH with significant activity and fibrosis, defined by an elevated NAFLD activity score ([NAS], NAS $\geq$ 4) and significant liver fibrosis (stage 2 fibrosis or higher [$\geq$F2]). It is an adaptation of NITs, utilizing the liver stiffness measurement (LSM) and controlled attenuation parameter (CAP) from VCTE, combined with AST.[9]

In the seminal paper by Newsome et al, the FAST score was internally validated in a derivation cohort of patients (350 patients with suspected NAFLD) with a satisfactory performance (C-statistic 0.80, 95% confidence interval [CI] 0.76–0.85). The model performed well in the external validation cohorts (C-statistic range 0.74–0.95, 0.85; 95% CI 0.83–0.87; n = 1026). A cutoff of $<$0.35 was proposed to achieve a sensitivity of 90% or greater to rule out patients with NAS $\geq$ 4 + NASH + F $\geq$ 2 and a cutoff of $\geq$ 0.67 to achieve a specificity of 90% or greater to rule in patients with NAS $\geq$ 4 + NASH + F $\geq$ 2. A positive predictive value (PPV) of 83% and a negative predictive value (NPV) of 85% were achieved in the derivation cohort, with PPV ranging from 33% to 81% and NPV from 73% to 100% in the external validation cohorts.[9]

The aim of our study was to assess the performance of the FAST score in accurately identifying NASH and significant fibrosis (NASH + $\geq$F2) in a nonclinical trial cohort of patients with histologically confirmed diagnosis of NASH. For this reason, we utilized the cutoff $\geq$0.35 as a rule in factor. We also compared the FAST score to other NITs and aimed to develop a model to identify additional risk factors to improve diagnostic accuracy.

Methods

Study Design and Characteristics of Patients

Patients aged $\geq$18 years with a histological diagnosis of NASH from January 2020 to June 2021 at a tertiary care hospital in New York, US, were identified through a retrospective chart review of the electronic medical record system. Patients were required to have corresponding VCTE data (LSM and CAP measurements) within 3 months of liver biopsy. Patients were excluded if they had additional causes of liver disease such as viral hepatitis, biliary obstruction, hepatocellular carcinoma, Wilson's disease, Budd Chiari Syndrome, autoimmune hepatitis, alcoholic liver disease or alcohol use ($>$20 g/day women, $>$30 g/day men), had AST or alanine aminotransferase (ALT) values $>$ 300 U/L, using steatogenic medications (amiodarone, methotrexate, tamoxifen, and corticosteroids), pregnant, or a history of liver transplantation. Liver biopsy slides were reviewed by a single pathologist who characterized diagnosis of NASH based on the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN), a composite score of steatosis, lobular inflammation, and hepatocyte ballooning with a nonalcoholic fatty liver disease activity score (NAS) of 4 and higher consistent with diagnosis of NASH. The study was approved by the Institutional Review Board of the Icahn School of Medicine at Mount Sinai and was in accordance with the Declaration of Helsinki.

Data Collection

Demographic data including age, gender, body-mass index (BMI), and ethnicity were collected. Presence of medical comorbidities were recorded for each individual. Laboratory data including aminotransferases (ALT, AST, alkaline phosphatase, gamma-glutamyl transferase, tests of liver function (albumin, platelet count, total bilirubin), and factors associated with metabolic comorbidities (eg, high density lipoprotein, low density lipoprotein, triglycerides (TG), and glycated hemoglobin A1C) were recorded. Severity of NAFLD determined by VCTE (stage of fibrosis estimated from LSM and steatosis grade estimated from CAP scores) within 3 months of liver biopsy was recorded. FAST score and FIB-4 were calculated using available laboratory data at the time of VCTE. LSM measurements were characterized as $<$8 kPa or $\geq$8 kPa. As we were interested in identifying those with significant fibrosis ($\geq$F2), we used LSM $\geq$8 kPa, FIB-4 $\geq$1.3, and FAST score $\geq$0.35.

Statistical Analysis

The primary analysis of our study was to evaluate the performance of the FAST score in predicting NASH + $\geq$F2. We assessed the performance of the FAST score by calculating the sensitivity, specificity, NPV, PPV, and overall accuracy when compared to histological evidence of NASH + $\geq$F2. This was repeated for LSM scores obtained from VCTE alone. Because we are solely assessing the ability to accurately identify all patients with NASH + $\geq$F2, we only report on the validation of the FAST score at $\geq$0.35 to correctly rule in and identify positive patients consistent with the literature.[9] We did not apply the cut-off of $>$0.67, which is consistent with risk of advanced fibrosis (stage 3 fibrosis and greater [$\geq$F3]). Differences in patient characteristics were assessed among those with NASH + $\geq$F2 and those with NASH + $<$F2 using student's t and chi-square tests. Univariate and backward entry multivariate logistic regression analysis were performed to identify risk factors in addition to FAST score $\geq$0.35 that are associated with accurate histological diagnosis of NASH + $\geq$F2.

Results

A total of 75 individuals with histological diagnosis of NASH were identified from January 2020 to June 2021. Majority of the patients were male (43/75, 57.3%), and

Table 1. Patient Demographics and Laboratory Data

Data points	Total population (NASH) N = 75 N (%) or mean (SD)	NASH + ≥F2 N = 56 N(%) or mean (SD)	NASH + <F2 N = 19 N(%) or mean (SD)	*P*-value[a]
Age (y)	49 (16)	51 (16)	43 (17)	.04
Males	43 (57.3%)	30 (54%)	13 (68%)	.26
Hispanic	38 (50.7%)	29 (52%)	9 (477%)	.69
Diabetes	28 (37.3%)	21 (38%)	7 (37%)	.05
Hypertension	28 (37.3%)	21 (38%)	7 (37%)	.55
BMI (kg/m^2)	32.75 (6.9)	33.1 (7.2)	31.6 (6.1)	.41
CAP score (dB/m)	319.73 (48.8)	321.1 (50.9)	315.8 (43.1)	.69
Fibroscan (kPa)	14.5 (11.1)	16.3 (12.1)	9.2 (4.7)	.001
AST (U/L)	68.5 (49.2)	73.7 (52.1)	53.4 (36.7)	.121
ALT (U/L)	97.8 (92.8)	93.2 (91.8)	111.5 (96.9)	.461
ALP (U/L)	116.5 (59.6)	112.4 (57.9)	128.6 (64.0)	.31
Albumin (g/dL)	4.11 (0.4)	4.1 (0.4)	4.3 (0.4)	.05
GGT (U/L)	135.3 (145.5)	136.5 (140.5)	131.4 (167.7)	.92
Total bilirubin (mg/dL)	0.73 (0.5)	0.76 (0.50)	0.65 (0.42)	.40
Platelets (mcL x 10^9)	221.72 (89.6)	219.9 (91.7)	227.2 (85)	.76
HDL (mg/dL)	45.53 (11.8)	43.9 (11.1)	50.1 (12.7)	.06
LDL (mg/dL)	105.08 (40.0)	103.6 (40.6)	109.3 (39.3)	.63
Triglycerides (mg/dL)	163.95 (94.2)	165.3 (84.4)	160.1 (120.8)	.85
FIB-4 index	2.06 (1.7)	2.4 (1.8)	1.2 (1.1)	.011
FAST score	0.62 (0.2)	0.66 (0.17)	0.49 (0.24)	.011

Data are presented as n (%) or mean (SD).
ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; HDL, high density lipoprotein; LDL, low density lipoprotein.
[a]Chi-square test *P*-values are presented for categorical variables, student's t-test *P*-values for continuous variables.

50.7% of individuals reported Hispanic ethnicity. The mean age was 49 years ± 16 years. The mean BMI was 32.75 ± 6.9 kg/m^2, consistent with class 1 obesity. The most common metabolic comorbidities were T2DM (50.7%) and hypertension (37.3%). The mean CAP score was 319.73 dB/m ± 48.8, consistent with severe steatosis, and mean LSM was 14.5 kPa ± 11.1 consistent with advanced fibrosis. Overall, our cohort was found to have a mean FAST score of 0.62 ± 0.2 and FIB-4 score of 2.06 ± 1.7, consistent with indeterminate liver fibrosis. Details of the demographic, clinical, and laboratory data of the study population are illustrated in Table 1.

Of the 75 individuals identified to have a histological diagnosis of NASH, we identified 56 individuals (74.3%) with NASH + ≥F2 and 19 individuals with NASH and less than F2 fibrosis. Descriptive baseline data for each cohort can be found in Table 1. Those with NASH + ≥F2 were 54% male, 52% Hispanic, and had an average BMI of 33.1, consistent with class 1 obesity. Mean baseline lab values reflected triglyceride level 165.3 mg/dL (84.4), ALT 93.2 U/L (91.8), and AST 73.7 U/L (52.1). The mean CAP score was 321.1 dB/m (50.9) consistent with severe steatosis and LSM was 16.3 kPa (12.1), consistent with advanced fibrosis (≥F3). All VCTE measurements were obtained with M probes. The mean FAST score is 0.7 (0.2) and FIB-4 is 2.4 (1.8).

Those with NASH + < F2 (n = 19) were found to be 68% male, 47% Hispanic, and mean BMI of 31.6kg/m^2(6.1), consistent with class 1 obesity. Mean baseline lab values reflected triglyceride levels of 160 mg/dL (120.8), ALT 111.5 U/L (96.9), and AST 53.4 U/L (36.7). The mean CAP score was 315.8 dB/m (43.1), consistent with severe steatosis and LSM of 9.2 kPa. The mean FAST score was 0.5 (0.2) and FIB-4 was 1.2 (1.1). When comparing those with significant fibrosis (n = 56) to those without (n = 19), there were statistically significant mean differences in age (51 vs 43 years, $P = .04$), LSM (16.3 kPa vs 9.2 kPa, $P = .001$), FAST score (0.66 vs 0.49, $P = .01$), and FIB-4 score (2.4 vs 1.2, $P = .01$).

Diagnostic Accuracy of FAST Score to Identify NASH and Significant Fibrosis

Cutoff of >0.35 to rule in NASH fibrosis. The FAST score cutoff of 0.35, as suggested by Newsome and colleagues[9] was assessed in its performance to identify NASH + ≥F2 fibrosis. We assessed the ability of the newly derived scoring to correctly identify NASH + ≥F2 fibrosis in the previously described cohort of 75 patients with histological indication of disease. The rule-out cutoff of ≥0.35 performed with a sensitivity of 96.4%, specificity of 36.8%, NPV of 77.7%, a PPV of 81.8%, and an overall accuracy of 81.3%.

After validating the performance of the FAST score ≥0.35, we assessed its performance in the presence of other NASH risk factors. Independent logistic regressions within this sample showed age (odds ratio (OR) = 0.05, CI:

Table 2. Shell Table for Validation Data

NASH diagnostic criteria/tests	Sensitivity	Specificity	PPV	NPV	Overall accuracy	C statistic	C statistic confidence interval
FAST ≥0.35	96.4%	36.8%	81.8%	77.7%	81.3%	0.70	(0.55, 0.84)
FIB-4 ≥1.3	64.3%	68.4%	86%	40%	65.0%	0.73	(0.59, 0.87)
LSM ≥8kPa	75%	52.6%	82.4%	41.6%	69.0%	0.72	(0.59, 0.85)
FAST ≥0.35 + age	94.6%	42.1%	82.7%	96.4%	81.3%	0.78	(0.64, 0.92)

1.0–1.07, $P = .05$) and a FAST ≥0.35 (OR = 15.75, CI: 2.9–85.48, $P = .001$) to be associated with correctly identifying NASH + ≥F2 based on histological evidence. The presence of T2DM ($P = .06$), hypertension ($P = .55$), LSM ≥8 kPa ($P = .051$), and BMI ($P = .40$) were not found to be statistically significant associations. A multivariable logistic regression ascertaining the effects of age on the predictability of the FAST score was statistically significant, $\chi 2(1) = 6.665$, $P = .010$. Patients with a FAST score of ≥0.35 have 23.8 times greater odds (OR = 23.8, CI: 3.97–142.89), and for every one year increase in age, a 5% increased odds of having NASH+ ≥F2 (OR = 1.05, CI: 1.01–1.09). The model explained 33.7% (Nagelkerke R2; demonstrating a moderate goodness of fit) of the variance in patients with histological indication of NASH but correctly classified 81% of cases.

Comparison of FAST Score to LSM and FIB-4

With the overall accuracy of both FAST and the newly proposed model, we assessed discrimination and overall accuracy utilizing the area under receiver operating characteristic (AUROC) curve procedure. We assessed the performance of the FAST score ≥0.35, and compared it to LSM ≥8 kPa and FIB-4 ≥1.3, all indicative of ≥F2 (Table 2). Although we found that FAST score ≥0.35 performed with the highest sensitivity (96.4%), it performed with the lowest c-statistic (0.70, CI: 0.55–0.84). LSM of ≥8 kPa performed marginally better (0.72, CI: 0.59–0.85), as did FIB-4 ≥1.3 (0.73, CI: 0.59–0.87). The FAST score overall performed better than LSM ≥8 kPa in correctly classifying patients with NASH + ≥F2 (overall accuracy 81.3% vs 69%). Lastly, the proposed FAST + Age model outperformed the others with a sensitivity of 94.6%, specificity of 42.1%, PPV of 82.7%, NPV of 96.4%, and c-statistic of 0.78 (CI: 0.64–0.92) in correctly identifying NASH + ≥F2 (Table 2; Figure).

Discussion

In this retrospective single-center study, we aimed to assess the performance of the FAST score in detecting NASH + ≥F2 utilizing real-world data and corresponding histological confirmation of NASH to validate the performance of a FAST score cutoff of ≥0.35. We compared its performance to that of LSM and FIB-4. We focused our analysis on a cutoff of ≥0.35, proposed by Newsome et al,[9] as we were interested in detecting individuals at risk of significant NASH or fibrotic NASH (NASH + NAS ≥4 + ≥F2) and did not assess the cutoff of 0.67, which was proposed as the cutoff for advanced fibrosis, which is consistent with ≥F3 fibrosis. We found that the FAST score had a reasonable diagnostic performance with an AUROC of 0.70, which further improved to 0.78 with age incorporated into the model. In addition, FAST score had similar performance to LSM and to FIB-4 for detecting individuals with NASH + ≥F2 (Table 2).

Our study results are consistent with the findings by Newsome et al,[9] who performed the original study deriving the FAST score and found an AUROC of 0.80 in the derivation cohort from England, 0.85 in the pooled validation cohort, and 0.86 in the USA screening cohort.[9] Data from our real-world experience cohort showed similar sensitivity (96.4%) and NPV (77.7%) when compared to the sensitivity (90%) and NPV (85%) of the original study by Newsome et al. In addition, the overall accuracy of our study (81.3%) was comparable to that of the original study (80%). Traditional risk factors such as T2DM and hypertension were not found to improve the accuracy of the FAST score, but interestingly, age was noted to increase the odds of NASH.

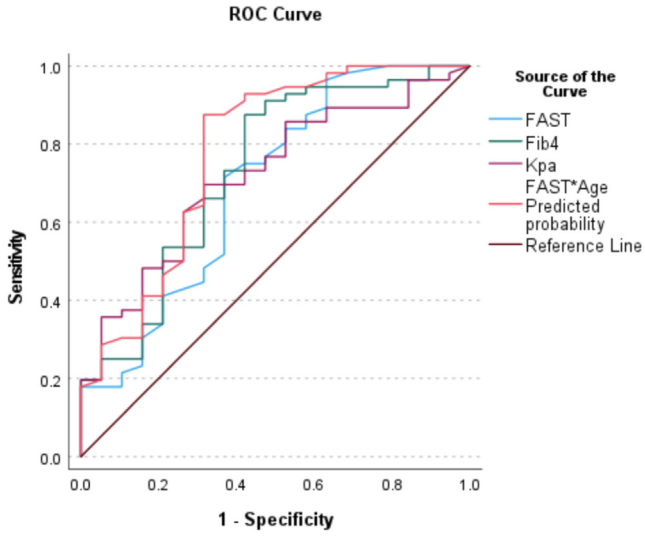

Figure. Comparison of different NIT cutoffs for F2 fibrosis and higher. A FAST score ≥0.35 performed with the highest sensitivity of 96.4%. The proposed FAST + age model outperformed the others with a sensitivity of 94.6% and specificity of 42.1% in correctly identifying NASH + ≥F2. kpa, kilopascal; ROC, receiver operating characteristic.

In a cross-sectional study of 287 patients in Brazil, the diagnostic performance of the FAST score using ≥0.35 cutoff to diagnose NASH + NAS ≥ 4 + ≥F2 revealed an AUROC of 0.78 (0.72–0.94) in the general population (average BMI 32 kg/m^2) and 0.81 (0.74–0.88) in patients with BMI > 32 kg/m^2.[10] In a recent analysis of 585 patients with biopsy-proven NASH from the multicenter NASH CRN Adult Database 2 cohort study, the AUROC of FAST score was 0.81 (95% CI: 0.77–0.84) for detection of NASH with NAS ≥4 + ≥F2 and had improved performance in non-whites vs whites (AUROC: 0.91 vs 0.78; $P = .001$) and in individuals with normal BMI vs BMI >35 kg/m^2 (AUROC: 0.94 vs 0.78, $P = .008$). Lastly, the FAST score had higher diagnostic accuracy than other NITs.[11] Our cohort was smaller than the referenced study but demonstrated similar performance.

Additionally, a study by Oeda et al evaluated the diagnostic accuracy of the FAST score in Japanese patients and compared it to FibroScan accuracy using two different probe sizes. In this study, no difference was detected between FibroScan and FAST scores when evaluating NASH with significant population.[12] Our study showed that FAST score performed similar to FibroScan, but with age incorporated into the model, the FAST score performed better (Table 2).

The diagnostic performance of a test depends on the population of interest, while predictive values depend on the prevalence of disease in a population. The findings from our cohort are notable since the population assessed is similar to both the derivation and validation cohorts in the original Newsome study: mean BMI consistent with class 1 obesity, T2DM, and hypertension being the most prevalent metabolic comorbidities, and >50% of the cohort was men. Interestingly, our cohort had higher mean ALT (68.5 U/L) and AST values (116.53 U/L), suggesting a common scenario for indication for a liver biopsy in the real world. The NPV of a test does not vary depending on the level of care (primary vs secondary) provided; thus, it is a strong indicator when evaluating the performance of the FAST score, which ultimately serves a purpose to assist in identifying patients suitable for clinical therapies and reduce unnecessary liver biopsies.

Sequential testing is an important methodology that can be used to increase sensitivity or specificity of disease detection. While there have not been any studies describing the role of sequential testing using the FAST score and other NITs, it is an important consideration that can be studied to improve diagnostic accuracy.

Our study has several strengths that are noteworthy. First, we demonstrated the ability to utilize the FAST score in the real world to improve the identification of NASH + ≥F2-fibrotic NASH. Secondly, we successfully compared the FAST score to other commonly used NITs such as FIB-4 and VCTE, equipping us with additional tools to stratify individuals at risk for NASH fibrosis. The cutoffs utilized for the NITs were reasonable and widely accepted to identify those with NASH + ≥F2. Lastly, though our patient population was small, the results of our study are generalizable to urban populations.

One of the unavoidable flaws of this study is the retrospective nature; hence, we were limited by the available data in the electronic medical record. However, the cohort was similar to the original validation cohort[9] and large NASH CRN study.[11] Secondly, the diagnosis of NASH fibrosis was determined by a single pathologist who reviewed each biopsy, which can be a source of observer bias. To address this, we reviewed the liver biopsy reports and applied the NASH CRN histological scoring system[11] for the diagnosis of NASH + ≥F2.

Conclusion

We validated the ability of the FAST score to detect NASH + ≥ F2 in the real world with a cutoff of ≥0.35. The FAST score performed reasonably well with an AUROC of 0.70, which further improved to 0.78 with age incorporated into the model.

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Correspondence:
Address correspondence to: Amreen M. Dinani, MD, FRCPC, Department of Gastroenterology and Hepatology, Duke University, 40 Duke Medicine Cir Clinic 2J, Durham, North Carolina 27710. e-mail: amreen.dinani@gmail.com.

Authors' Contributions:
Amreen M. Dinani: Conception and design, administrative support, provision of study material or patients, collection and assembly of data, data analysis and interpretation, manuscript writing, final review of manuscript. Gres Karim: Provision of study material or patients, collection and assembly of data, data analysis and interpretation, manuscript writing, final review of manuscript. Dewan Giri: Provision of study material or patients, collection and assembly of data, data analysis and interpretation, manuscript writing, final review of manuscript. Brooke Wyatt: Provision of study material or patients, collection and assembly of data, data analysis and interpretation, manuscript writing, final review of manuscript.

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Reporting Guidelines:
Declaration of Helsinki.