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Noninvasive assessment of liver disease severity in patients with nonalcoholic fatty liver disease (NAFLD) and type 2 diabetes

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Abstract

Background and Aims: We evaluated the diagnostic accuracy of simple, noninvasive tests (NITs) in NAFLD patients with type 2 diabetes (T2D).

Methods and Results: This was an individual patient data meta-analysis of 1780 patients with biopsy-proven NAFLD and T2D. The index tests of interest were FIB-4, NAFLD Fibrosis Score (NFS), aspartate aminotransferase-to-platelet ratio index, liver stiffness measurement (LSM) by vibration-controlled transient elastography, and AGILE 3+. The target conditions were advanced fibrosis, NASH, and fibrotic NASH(NASH plus F2-F4 fibrosis). The diagnostic performance of noninvasive tests. individually or in sequential combination, was assessed by area under the receiver operating characteristic curve and by decision curve analysis. Comparison with 2278 NAFLD patients without T2D was also made. In NAFLD with T2D LSM and AGILE 3+ outperformed, both NFS and FIB-4 for advanced fibrosis (area under the receiver operating characteristic curve:LSM 0.82, AGILE 3 + 0.82, NFS 0.72, FIB-4 0.75, aspartate aminotransferase-to-platelet ratio index 0.68; $p < 0.001$ of LSM-based versus simple serum tests), with an uncertainty area of 12%–20%. The combination of serum-based with LSM-based tests for advanced fibrosis led to a reduction of 40%–60% in necessary LSM tests. Decision curve analysis showed that all scores had a modest net benefit for ruling out advanced fibrosis at the risk threshold of 5%–10% of missing advanced fibrosis. LSM and AGILE 3+ outperformed both NFS and FIB-4 for fibrotic NASH (area under the receiver operating characteristic curve:LSM 0.79, AGILE 3+ 0.77, NFS 0.71, FIB-4 0.71; $p < 0.001$ of LSM-based versus simple serum tests). All noninvasive scores were suboptimal for diagnosing NASH.

Conclusions: LSM and AGILE 3+ individually or in low availability settings in sequential combination after FIB-4 or NFS have a similar good diagnostic

Abbreviations: ALT, alanine transaminase; APRI, aspartate aminotransferase-to-platelet ratio index; AUROC, area under ROC curve; BMI, body mass index; DCA, decision curve analysis; IPDMA, individual patient data meta-analysis; LSM, liver stiffness measurement; NFS, NAFLD Fibrosis Score; NPV, negative predictive value; PPV, positive predictive value; T2D, type 2 diabetes.

Grazia Pennisi and Marco Enea equally contributed to the study.

This individual patient data meta-analysis is being conducted as part of the imaging study in the LITMUS (Liver Investigation: Testing Marker Utility in Steatohepatitis) project.

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accuracy for advanced fibrosis and an acceptable diagnostic accuracy for fibrotic NASH in NAFLD patients with T2D.

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INTRODUCTION

NAFLD, affecting roughly 25% of the general adult population,[1] is a leading cause of chronic liver disease.[2] NAFLD complications[3,4] and the severity of liver fibrosis are the main drivers of prognosis in NAFLD, with more severe liver fibrosis incurring a higher risk of developing liver-related events (HCC and liver decompensation) and extrahepatic events (mostly cardiovascular events and extrahepatic cancer).[5,6]

NAFLD and type 2 diabetes (T2D) have a complex bidirectional interplay: NAFLD increases the risk of T2D development,[7] and T2D is a risk factor for NAFLD occurrence, severity, and progression toward liver cirrhosis and its complications.[8] Consequently, the estimated prevalence of NAFLD in people with T2D is about 55%, and—most relevant—NASH and advanced fibrosis can be observed in about 37% and 17%, respectively, of patients with T2D.[9]

The high prevalence of NAFLD and NAFLD-related liver damage in patients with T2D led clinical guidelines to encourage screening for advanced fibrosis in patients with metabolic dysfunctions, including those with T2D.[10,11] For this purpose, noninvasive scores, like FIB-4 and NAFLD Fibrosis Score (NFS), and liver stiffness measurement (LSM) by vibration-controlled transient elastography (VCTE) have been largely validated as accurate tools to exclude advanced fibrosis in NAFLD,[12,13] and their rational use is recommended by international guidelines.[10] However, preliminary evidence suggests a poor accuracy of these scores/tools in patients with T2D,[12,14,15] finally leading to high referral rates for expert evaluation.[16] Moreover, the AGILE 3 +score, based on the simultaneous combination of aspartate aminotransferase (AST)/alanine transaminase (ALT) ratio, platelet count, T2D status, sex, age, and LSM by VCTE, has been recently developed and proposed for the diagnosis of advanced fibrosis in NAFLD,[17,18] but data on its performance in the diabetic population are lacking. On the other side, the identification of NAFLD patients with NASH, and—most relevant—with fibrotic NASH, especially in high-risk groups like patients with T2D, is an important need for inclusion in phase 2b and phase 3 clinical trials assessing pharmacological treatment of NASH patients.[19,20]

Our aim was thus to explore the diagnostic accuracy of simple serum-based noninvasive scores and LSM by VCTE for the diagnosis of advanced fibrosis, NASH, and fibrotic NASH in a large cohort of patients with histological diagnosis of NAFLD and T2D. A comparison with NAFLD patients without T2D was also made.

METHODS

Patients

For the present study, we used the subgroup of 1780 patients with histological diagnosis of NAFLD and T2D from a previously published individual patient data meta-analysis of 37 studies, which aimed to assess the accuracy of LSM by VCTE and noninvasive scores for ruling out advanced fibrosis in biopsy-confirmed NAFLD patients.[12] All authors who had provided data for the original individual patient data meta-analysis (IPDMA) were contacted with details of the present study, and their data were only included with their agreement. In the present analysis, we considered all but 5 studies included in the IPDMA[21–25] because the authors of those 5 studies have not responded to the email that was asking for their consent for participating in this subanalysis. Search details, inclusion criteria, and quality assessment of the studies were reported in the original study;[12] literature search for eligible studies for this IPDMA stopped at April 2020.

Briefly, studies reporting data on adults (18 y or above) with NAFLD after the exclusion of other causes of liver diseases and paired liver histology and LSM by VCTE were eligible. All studies were considered if the interval of time between liver biopsy, LSM, and noninvasive scores was within 6 months. The diagnosis of T2D was made according to the American Diabetes Association,[26] using a value of fasting blood glucose $\geq$ 126 mg/dL or based on the use of antidiabetic therapy. In patients with a previous diagnosis of T2D, current medications were documented. Finally, only studies reporting histological classification of liver fibrosis based on the NASH Clinical Research Network staging system[27] were considered.

Patients and the public were not involved in the conduct of this study as there was no direct patient participation in the study. All research was conducted in accordance with both the Declarations of Helsinki and Istanbul and was approved by each appropriate ethics and/or institutional review committee(s).

Assessment of liver histology

Liver histology was based on local reporting from the original studies based on the NASH Clinical Research Network staging system.[27] NASH was defined by the presence of a NASH activity score >3 with at least grade 1 in each component; fibrotic NASH was defined by the presence of NASH plus fibrosis stage F2-F4; advanced fibrosis was defined by the presence of fibrosis stage F3-F4.

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Noninvasive assessment of liver fibrosis

The FIB-4 (comprising age, AST, ALT, and platelets [PLT]) score was calculated using the original reported formula, and patients were classified as low risk of advanced fibrosis if FIB-4 <1.30, intermediate risk if FIB-4 was between 1.30 and 2.67, and high risk if FIB-4 >2.67.[28]

The NFS (comprising age, body mass index (BMI), AST, ALT, albumin, PLT, and T2D status) score was calculated using the original reported formula, and patients were classified as low risk of advanced fibrosis if NFS <−1.455, intermediate risk if NFS was between −1.455 and 0.675, and high risk if NFS >0.675.[29]

The AST-to-platelet ratio index (APRI) was also computed.[30] Vibration-controlled transient elastography was performed with the FibroScan (Echosens, Paris, France) medical device. For this meta-analysis, if only 1 VCTE-based LSM was available, then this was included in the main analysis irrespective of probe type and BMI. Where 2 VCTE-based LSM were available (1 with each probe), the main analysis included the M probe measurement for BMI <30 kg/m^2 and the XL probe measurement for BMI ≥30 kg/m^2. Therefore, all LSM cutoffs were determined independent of probe type. LSM <7.9 kPa was defined as indicating a low risk of F3-F4 fibrosis; LSM 7.9–9.6 kPa as an intermediate risk; LSM >9.6 kPa as a high risk.[31]

The AGILE 3+ score (comprising age, sex, AST, ALT, PLT, T2D status, and LSM) was calculated using the original reported formula, and patients were classified as low risk of advanced fibrosis if AGILE 3+ <0.45, intermediate risk if AGILE 3+ was between 0.45 and 0.67, and high risk if AGILE 3+ ≥0.68.[17,18]

Statistics

Data for continuous variables were expressed as mean, and SD or median and interquartile range, and data for categorical variables were expressed as frequency and percentage. Differences between continuous data were assessed by the Student *t* test or by the Mann-Whitney *U* test. Differences between categorical variables were assessed by the χ^2 test.

The accuracy of each score for the detection of advanced fibrosis (F3-F4), NASH, and fibrotic NASH (NASH plus F2-F4 fibrosis) was assessed using the area under the receiver operating characteristic curves described as area under the receiver operating characteristic curve (AUROC). AUROCs were compared using De Long test statistic. Cutoff points of LSM, NFS, FIB-4, and AGILE 3 + for the advanced fibrosis model were derived from the literature. Specific cutoffs with sensitivity >90% for ruling out or specificity >90% for ruling in all outcomes were calculated, and for this purpose, the cohort was split in into a training (cohorts with ≥100 enrolled patients) and a validation (cohorts with <100 enrolled patients). Accordingly, false-negative and false-positive rates of the single test, as well as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were calculated.

Finally, we also evaluated the accuracy of sequential combination strategies based on FIB-4 or NFS as a first test, and LSM or AGILE 3+ as a second test in those with FIB-4 or NFS values higher than the rule out cutoffs.

The main analysis was conducted to maximize data for each noninvasive test (NIT). For a valid comparison of the performance of NITs, a separate analysis was conducted in the subgroup of patients with a complete data set.

Subgroup analysis was performed according to age (<35, 35–65, >65 y), BMI (<30 kg/m^2, ≥30 kg/m^2), and aminotransferase levels (normal vs. abnormal ALT). For this last subgroup analysis, the upper limit of normal for ALT is 19 IU/L for women and 30 IU/L for men.[32] We also evaluated the overall accuracy of LSM in the subgroup of patients with BMI <30 kg/m^2 measured with the M probe and with BMI ≥30 kg/m^2 measured with XL probe.

As AUROC focuses only on the predictive accuracy of a model, not considering cases where a false-negative result is more harmful than a false-positive result, we also performed a decision curve analysis (DCA) for identifying threshold probabilities at which the use of noninvasive criteria will translate into the maximum net benefit of detecting advanced fibrosis.[33,34]

DCA evaluated prediction models in comparison with default strategies of performing liver biopsy in all patients or none allowing an assessment of the overall yield of prediction rules. DCA estimates a “net benefit” for each prediction rule, defined as where *w* is the odds of true diagnosis (ie, advanced fibrosis in this case) across different threshold probabilities. In this setting, net benefit represents a composite of the benefit gained by performing liver biopsy for truely advanced fibrosis in patients classified as high risk according to noninvasive scores (true positive) and risk/discomfort incurred due to liver biopsy in those without advanced fibrosis but who were classified as high risk according to noninvasive scores (false positive). Threshold probability represents a theoretical risk level where the expected benefit of treatment is equal to the expected risk of avoiding treatment (eg, the benefit of liver biopsy equals the risk of not performing it). Thus, the net benefit is assessed across a range of threshold probabilities to identify the best diagnostic strategy for different risk-scenarios.

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All data were analyzed using R Studio. DCA was implemented in R using code derived from Zhang et al.[35,36] In addition to the base packages in R, tidy verse, survival, survminer, boot, reshape2, and readxl packages were used.

RESULTS

Features of patients with NAFLD and T2D

Baseline characteristics of the 1780 patients with NAFLD and T2D stratified for advanced fibrosis, NASH, and fibrotic NASH are shown in Supplemental Table S1 http://links.lww.com/HEP/E554.

LSM was determined in 1692 (95%), FIB-4 in 1681 (94%), NFS in 1001 (56%), and AGILE 3+ in 1603 (90%.) patients. Overall, 46.2% of patients had advanced fibrosis, 77.4% of patients had NASH, and 55.8% of patients had fibrotic NASH.

Supplemental Tables S2 and S3 (http://links.lww.com/HEP/E554) report baseline characteristics of the 748 patients with a complete data set.

Diagnostic accuracy of noninvasive scores/tools for advanced fibrosis in patients with NAFLD and T2D

LSM, FIB-4, NFS, APRI, and AGILE 3+ had AUROCs of 0.82, 0.75, 0.72, 0.68, and 0.82 for advanced fibrosis (Table 1, Figure 1A). FIB-4 had a similar acceptable diagnostic accuracy as NFS ($p = 0.30$) and worked significantly better than APRI ($p < 0.001$). LSM and AGILE 3+ had good performance and performed similarly ($p = 0.60$) and significantly better than all serum-based tests ($p < 0.001$ for all comparisons). These results were confirmed when performing a head-to-head comparison in the cohort with a complete data set (Supplemental Figure S1A, http://links.lww.com/HEP/E555 and Supplemental Table S4, http://links.lww.com/HEP/E554).

Considering the poor accuracy of APRI, further analyses no longer consider this score.

Analyses considering cutoffs from the literature for the diagnosis of advanced fibrosis are reported in Table 2. Proportions of patients classified as having a low, intermediate, and high risk of advanced fibrosis were 46%, 40%, and 14% by using FIB-4; 27.6%, 55.8%, and 16.6% by using NFS; 35.8%, 12.9%, and 51.2% by using LSM; and 36.4%, 20.1%, and 43.5%, by using AGILE 3+, respectively. Consequently, FIB-4 had the highest proportion of patients at low risk of advanced fibrosis, and LSM had the lowest proportion of patients falling into the uncertainty area. NFS and LSM had the highest sensitivity (88%), LSM the highest NPV (84%), FIB-4 the highest specificity (93%), and the highest PPV (75%) (Table 2). Similar results were observed when comparing the scores in the cohort with a complete data set (Supplemental Table S5, http://links.lww.com/HEP/E554).

We further evaluated the performance of LSM, FIB-4, NFS, and AGILE 3+ to diagnose advanced fibrosis in sequential combinations. When selecting threshold combinations for FIB-4 (<1.3) and NFS (<-1.455) available in the literature and pairing them with the best threshold pair for LSM (<7.9 and ≥ 9.6 kPa) or AGILE 3+ (<0.45 and ≥ 0.68), the FIB-4→LSM strategy lead to the highest proportion of patients identified as being at low risk of advanced fibrosis, and the NFS→LSM strategy to the lowest proportion of patients falling in the uncertainty area (Table 3). Furthermore, NFS→LSM and NFS→AGILE 3+ strategies lead to the highest sensitivity (79% and 80%, respectively) and NPV (84% and 82%, respectively), whereas the FIB-4→LSM and FIB-4→AGILE 3+ strategies lead to the highest specificity (86% and 84%,

TABLE 1 Comparison in the entire population of NAFLD patients with T2D of AUCs of LSM and different scores for diagnosing F3-F4 fibrosis. NASH and fibrotic NASH

	F3-F4 fibrosis		NASH		Fibrotic NASH	
	AUC	*p*	AUC	*p*	AUC	*p*
APRI vs. NFS	0.68–0.72	0.163	0.70–0.66	0.844	0.70–0.71	0.879
APRI vs. FIB4	0.68–0.75	<0.001	0.70–0.65	0.054	0.70–0.71	0.879
APRI vs. LSM	0.68–0.82	<0.001	0.70–0.71	0.883	0.70–0.79	0.001
APRI vs. AGILE 3+	0.68–0.82	<0.001	0.70–0.69	0.883	0.70–0.77	0.019
NFS vs. FIB4	0.72–0.75	0.304	0.66–0.65	0.883	0.71–0.71	0.879
NFS vs. LSM	0.72–0.82	<0.001	0.66–0.71	0.507	0.71–0.79	0.004
NFS vs. AGILE 3+	0.72–0.82	<0.001	0.66–0.69	0.883	0.71–0.77	0.055
FIB4 vs. LSM	0.75–0.82	<0.001	0.65–0.71	0.317	0.71–0.79	0.004
FIB4 vs. AGILE 3+	0.75–0.82	<0.001	0.65–0.69	0.883	0.71–0.77	0.058
LSM vs. AGILE 3+	0.82–0.82	0.608	0.71–0.69	0.883	0.79–0.77	0.879

Abbreviations: APRI, AST-to-platelet ratio index; LSM, Liver stiffness measurement; NFS, NAFLD fibrosis score.

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respectively) and PPV (79% and 76%, respectively) (Table 3). Similar results were observed when comparing the scores in the cohort with a complete data set (Supplemental Table S6, http://links.lww.com/HEP/E554).

The net benefit of FIB-4, NFS, LSM, and AGILE 3+ scores for ruling out advanced fibrosis at 5%, 10%, and 15% threshold probabilities of missing advanced fibrosis is shown in Figure 2. At the risk thresholds of 5% and 10% of missing advanced fibrosis, all scores/tools showed no benefit for ruling out advanced fibrosis compared with the strategy of performing a liver biopsy in all patients, whereas at the risk threshold of 15%, the observed net benefit was modest and LSM outperformed AGILE 3+, NFS, and FIB-4. The results obtained for ruling in advanced fibrosis are shown in Supplemental Figure S2 (http://links.lww.com/HEP/E555). When considering strategies based on the combination of FIB-4 or NFS with LSM or AGILE 3+, these showed no benefit for ruling out advanced fibrosis at the risk thresholds of 5% and 10% of missing advanced fibrosis (Supplemental Figure S3, http://links.lww.com/HEP/E555).

Identification of best cutoffs for advanced fibrosis in patients with NAFLD and T2D

Considering the unique opportunity to have a large cohort of patients with histological diagnosis of NAFLD and T2D, we split the population into a training and a validation set to search for the best rule out and rule in cutoffs for advanced fibrosis. Differences between training and validation cohorts are reported in Supplemental Table S7 (http://links.lww.com/HEP/E554).

These analyses are reported in Table 4. Notably, the accuracy of the new proposed cutoffs was replicated in the validation set where NPV and PPV of about 80% were maintained at the cost of an uncertainty area of about 35%–38% for LSM and AGILE 3+, and of about 45%–58% for NFS and FIB-4 (Table 4).

Net benefit of FIB-4, NFS, LSM, and AGILE 3+ scores by using these new cutoffs for ruling out advanced fibrosis at 5%, 10%, and 15% threshold probabilities of missing advanced fibrosis is showed in Figure 3A.

Comparison of diagnostic accuracy of noninvasive scores/tools for advanced fibrosis between NAFLD patients with or without T2D

The baseline characteristics of the 2278 NAFLD patients with T2D arising from the same studies considered in this IPDMA, respect to NAFLD patients without T2D are shown in Supplemental Table S8 (http://links.lww.com/HEP/E554).

Supplemental Table S9 (http://links.lww.com/HEP/E554) reports the comparison of AUROCs of LSM, FIB-4, NFS, and AGILE 3+ according to T2D status. All noninvasive scores performed similarly in NAFLD patients with T2D compared with those without for predicting advanced fibrosis (Supplemental Table S9, http://links.lww.com/HEP/E554).

When considering cutoffs from the literature for the diagnosis of advanced fibrosis, in nondiabetic patients with respect to population with NAFLD and T2D, noninvasive scores had lower sensitivity—except for FIB-4, which was similar—but higher specificity and presented a lower uncertainty area—except for LSM that was similar (Supplemental Table S10, http://links.lww.com/HEP/E554).

The diagnostic accuracy of LSM, FIB-4, NFS, and AGILE 3+ in sequential combinations is reported in Supplemental Table S11 (http://links.lww.com/HEP/E554). With respect to NAFLD patients with T2D, in nondiabetics the sequential combination of FIB-4 or NFS with LSM or AGILE 3+ generated a lower uncertainty area and higher specificity but lower sensitivity, especially for NFS-based algorithms (Supplemental Table S11, http://links.lww.com/HEP/E554).

The net benefit of FIB-4, NFS, LSM, and AGILE 3+ scores alone or in combination for ruling out advanced fibrosis at 5%, 10%, and 15% threshold probabilities of missing advanced fibrosis is shown in Supplemental Figure S4 (http://links.lww.com/HEP/E555). With respect to the population of NAFLD with T2D, the net benefit for ruling out advanced fibrosis at 5%,10%, and 15% risk threshold was higher for all noninvasive scores.

The results of the search for best rule out and rule in cutoffs for advanced fibrosis are reported in Supplemental Table S12 (http://links.lww.com/HEP/E554), Supplemental Figure S5 (http://links.lww.com/HEP/E555), and Supplemental Table S13 (http://links.lww.com/HEP/E554). With respect to the diabetic population, best rule in and rule out cutoffs for nondiabetic patients generated a smaller uncertainty area; FIB-4 best cutoffs were similar between diabetic and nondiabetic patients, whereas rule in cutoff for LSM was lower in nondiabetic compared with diabetic patients.

Diagnostic accuracy of noninvasive scores/tools for NASH and fibrotic NASH in patients with NAFLD and T2D

LSM, FIB-4, NFS, APRI, and AGILE 3 + had corresponding AUROCs of 0.71, 0.65, 0.66, 0.70, and 0.69 for identifying NASH (Table 1, Figure 1B), and of 0.79, 0.71, 0.71, 0.70, and 0.77 for fibrotic NASH (Table 1, Figure 1C). Consistently, all noninvasive tools tested

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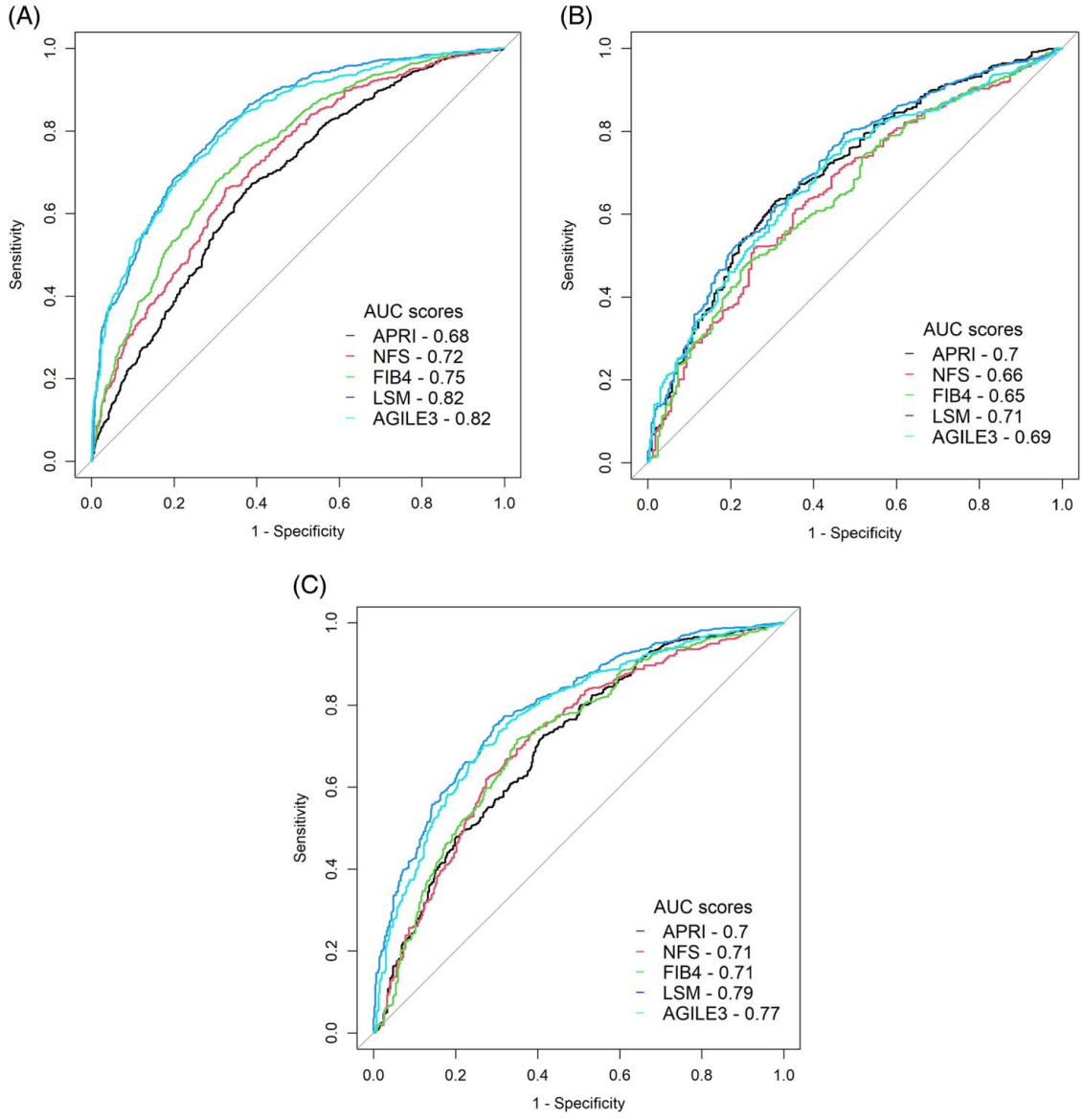

FIGURE 1 ROC curves for prediction of F3-F4 fibrosis (A), NASH (B), and fibrotic NASH (C) using APRI, NFS, FIB-4, LSM, and AGILE 3+ in NAFLD patients with diabetes.

here poorly predicted the presence of NASH, whereas LSM and AGILE 3+ have acceptable accuracy for detecting fibrotic NASH, LSM being significantly better than all the other simple serum-based tests ($p < 0.01$ for all) and with a similar performance as AGILE 3 + ($p = 0.87$). These trends were confirmed when performing a head-to-head comparison of LSM, FIB-4, NFS, and AGILE 3 + in the cohort with a complete data set (Supplemental Table S4, http://links.lww.com/HEP/E554).

The best rule out and rule in cutoffs for NASH as well as their operating characteristics are reported in Supplemental Table S14 (http://links.lww.com/HEP/E554). All scores/tools showed no benefit in ruling out NASH (Figure 3B).

Table 4 shows the best rule out and rule in cutoffs for fibrotic NASH and their operating characteristics in both training and validation sets. LSM identified 1 patient in three fourth as at high risk of fibrotic NASH at a specificity and a PPV of 91% and 80%, respectively, leading to an uncertainty area of 45.3%. At the risk threshold of 5%, 10%, and 15% of missing fibrotic NASH, all noninvasive scores had no benefit for ruling out fibrotic NASH (Figure 3C).

Comparison of diagnostic accuracy of noninvasive scores/tools for NASH and fibrotic NASH between patients with NAFLD with or without T2D

All noninvasive scores performed similarly poor for diagnosing NASH in both diabetic and nondiabetic cohorts. Otherwise, their accuracy for predicting fibrotic

TABLE 2 Diagnostic accuracy in terms of sensitivity, specificity, PPV, NPV, and 95% CIs of LSM and different scores for diagnosing advanced fibrosis (F3-F4) by using literature suggested cutoffs in the entire population of NAFLD patients with T2D

	STAGC				
	Sensitivity	Specificity	PPV	NPV	Uncertainty area (%)
NFS					
< −1.455 (n = 276; 27.6%)	0.88 (0.84–0.91)	0.38 (0.34–0.42)	0.50 (0.46–0.53)	0.82 (0.77–0.87)	55.8
> 0.676 (n = 166; 16.6%)	0.27 (0.3–0.32)	0.91 (0.88–0.93)	0.67 (0.60–0.75)	0.65 (0.61–0.68)	—
FIB4					
< 1.3 (n = 773; 46%)	0.73 (0.69–0.76)	0.62 (0.59–0.65)	0.62 (0.59–0.66)	0.72 (0.69–0.75)	40
> 2.67 (n = 236; 14%)	0.23 (0.20–0.26)	0.93 (0.92–0.95)	0.75 (0.69–0.80)	0.58 (0.56–0.61)	—
LSM					
< 8 kPa (n = 606; 35.8%)	0.88 (0.86–0.90)	0.56 (0.53–0.60)	0.64 (0.61–0.66)	0.84 (0.81–0.87)	12.9
> 9.6 kPa (n = 867; 51.2%)	0.77 (0.74–0.80)	0.71 (0.68–0.74)	0.70 (0.66–0.73)	0.78 (0.75–0.81)	—
AGILE 3+					
< 0.45 (n = 583; 36.4%)	0.87 (0.84–0.89)	0.56 (0.53–0.60)	0.63 (0.60–0.66)	0.83 (0.80–0.86)	20.1
> 0.68 (n = 698; 43.5%)	0.69 (0.65–0.72)	0.78 (0.76–0.81)	0.73 (0.70–0.77)	0.74 (0.71–0.77)	—

Abbreviations: NFS, NAFLD fibrosis score; LSM, liver stiffness measurement.

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NASH was significantly better in nondiabetic compared with diabetic patients (Supplemental Table S9, http://links.lww.com/HEP/E554).

With respect to the diabetic population, best rule in and rule out cutoffs for fibrotic NASH in nondiabetic patients generated a smaller uncertainty area for LSM; FIB-4 best cutoffs were similar between diabetic and nondiabetic patients, whereas rule in cutoff for LSM was lower in nondiabetic compared with diabetic patients (Supplemental Table S12 http://links.lww.com/HEP/E554 and Supplemental Figure S5).

Subgroup analyses in patients with NAFLD and T2D

Subgroup analyses for the diagnosis of advanced fibrosis, NASH and fibrotic NASH are reported in Table 5. When looking at the diagnosis of advanced fibrosis, all scores/tools had a trend for better accuracy in patients older than 35 years and performed significantly better in nonobese patients; FIB-4 and AGILE 3+ had significantly higher accuracy in patients with normal ALT, whereas in the same subgroups, NFS

TABLE 3 Diagnostic accuracy in terms of sensitivity, specificity, PPV, NPV, and 95% CIs of combination of FIB-4 or NFS with LSM or AGILE 3+ for diagnosing advanced fibrosis (F3-F4) in the entire population of NAFLD patients with T2D by using literature suggested cutoffs

	Se	Sp	PPV	NPV	Uncertainty area (%)
FIB4 → LSM					
Rule out (n = 974; 59.5%)	0.66 (0.62–0.69)	0.81 (0.78–0.84)	0.75 (0.71–0.78)	0.74 (0.71–0.76)	16
Rule in (n = 566; 34.5%)	0.59 (0.56–0.63)	**0.86** (0.84–0.89)	**0.79** (0.75–0.82)	0.71 (0.68–0.74)	—
NFS → LSM					
Rule out (n = 481; 51.7%)	**0.79** (0.75–0.83)	0.72 (0.69–0.76)	0.66 (0.61–0.70)	**0.84** (0.80–0.87)	**8.2**
Rule in (n = 374; 40.2%)	0.70 (0.65–0.75)	0.80 (0.76–0.83)	0.70 (0.65–0.75)	0.80 (0.76–0.83)	—
FIB4 → AGILE 3+					
Rule out (n = 874; 53.3%)	0.68 (0.65–0.72)	0.72 (0.69–0.75)	0.67 (0.64–0.71)	0.73 (0.70–0.76)	10
Rule in (n = 601; 36.7%)	0.61 (0.57–0.64)	**0.84** (0.81–0.86)	**0.76** (0.72–0.79)	0.71 (0.69–0.74)	—
NFS → AGILE 3+					
Rule out (n = 434; 46.6%)	**0.80** (0.75–0.84)	0.64 (0.60–0.68)	0.60 (0.56–0.64)	**0.82** (0.79–0.86)	15.1
Rule in (n = 357; 38.3%)	0.67 (0.62–0.71)	0.81 (0.77–0.84)	0.70 (0.65–0.75)	0.78 (0.75–0.82)	—

Note: Used cutoffs: FIB-4 rule out <1.30, rule in > 2.67; NFS rule out <−1.455, rule in > 0.675; LSM rule out <7.9 kPa, rule in > 9.6 kPa; AGILE 3+ rule out <0.45, rule in ≥ 0.68. Bold characters indicate the best value for each category.
Abbreviations: NFS, NAFLD fibrosis score; LSM, Liver stiffness measurement.

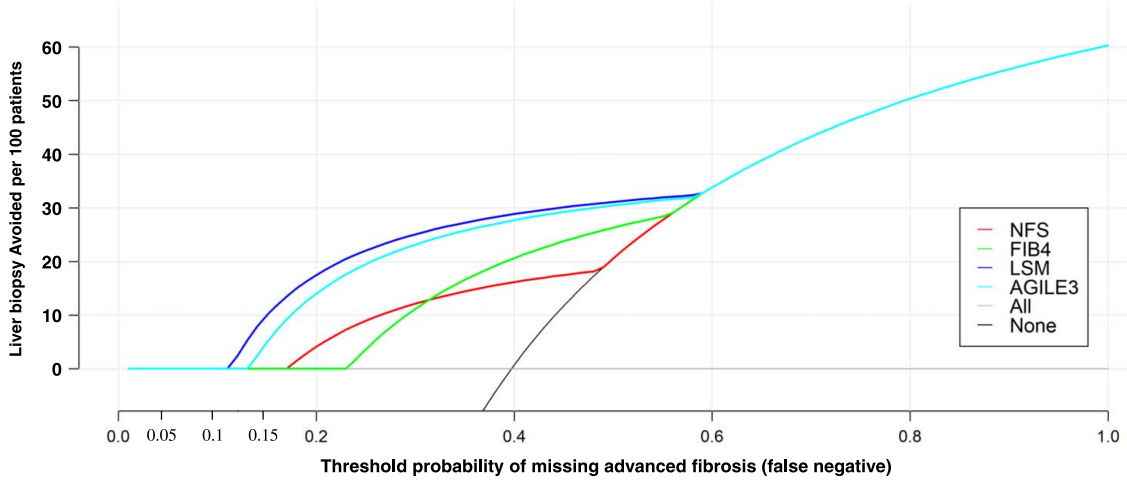

FIGURE 2 Net benefits by decision curve analyses of NFS, FIB-4, LSM, and AGILE 3+ for ruling out advanced liver fibrosis in NAFLD patients with diabetes.

and LSM had a nonsignificant trend for better performance (Table 5). Finally, looking at the diagnosis of fibrotic NASH, the only significant difference was for a higher accuracy of LSM in patients with normal ALT.

Finally, when looking at patients where LSM was performed by using the M probe in nonobese and the XL probe in obese patients, the overall accuracy for advanced fibrosis, NASH, and fibrotic NASH was 0.86, 0.72, and 0.81 for LSM, and 0.85, 0.71, and 0.80 for AGILE 3+; these results were similar to those obtained in the entire cohort. In this subgroup, we confirmed a higher accuracy of LSM in nonobese patients compared with obese patients (AUROCs 0.865 vs. 0.802, $p = 0.04$).

DISCUSSION

In this study on a large cohort of patients with histological diagnosis of NAFLD and T2D, we provided evidence that LSM and AGILE 3+ have a good diagnostic accuracy for advanced fibrosis and an acceptable diagnostic accuracy for fibrotic NASH, whereas AGILE 3+ did not provide any additional relevant diagnostic insights over and above LSM alone. Overall, both LSM and AGILE 3+ outperformed FIB-4 and NFS, which showed an acceptable performance. The sequential combination of serum-based tests with LSM-based tests for advanced fibrosis allowed to limit the number of LSM-based tests—mostly with FIB-4. Furthermore, DCA showed that the net benefit for the ruling out advanced fibrosis and fibrotic NASH was modest for all tools. In comparison to NAFLD patients without T2D, the overall accuracy of NITs for advanced fibrosis was similar even if with a lower net benefit mainly related to lower specificity and higher uncertainty area, whereas the accuracy for fibrotic NASH was lower.

In this large IPDMA on patients with NAFLD and T2D, simple serum-based tests lead to a high uncertainty area ranging from 40% for FIB-4 to 55% for NFS, the latter leading to the highest sensitivity (88%) and FIB-4 to the highest specificity (93%). Otherwise, LSM and AGILE 3+ were characterized by a low uncertainty area ranging from 12% to 20%, with highest sensitivity of 88% for LSM and highest specificity of 78% for AGILE 3+. Overall, these data, according to European and American guidelines,[10,11] suggest, when available and in a tertiary setting, to use LSM-based tests as first tests while demanding the use of simple serum scores where LSM is not available. AGILE 3+ can be an alternative to LSM but, at least in a diabetic population, does not provide any additional relevant diagnostic insights. We also tested the strategy of using serum-based scores as triage and to refer for LSM-based tests when patients were at intermediate-to-high risk by simple serum-based scores. This strategy led to a relevant reduction in the proportion of patients to an uncertainty area ranging from 8% to 16%, keeping the highest sensitivities for NFS-based algorithms (79%–80%) and the highest specificities for FIB-4-base algorithms (84%–86%). These results confirm that the sequential combination strategies can be useful also in the setting of NAFLD with T2D, even a big proportion of patients (about 50% for FIB-4 and about 70% for NFS) is worthy of being referred for LSM-based assessment. From a clinical point of view, FIB-4–based strategies may be preferred because they can spare more LSM compared with NFS (Supplemental Figure S6, http://links.lww.com/HEP/E555). Notably, in our decision curve analysis, noninvasive tests and their combinations showed similar modest net benefit for ruling out advanced fibrosis at threshold probabilities of 5% and 10% of missing advanced fibrosis.

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TABLE 4 Diagnostic accuracy for advanced fibrosis and fibrotic NASH in terms of sensitivity, specificity, PPV, NPV, and 95% CIs of NFS, FIB-4, LSM, and AGILE 3+ in the training and validation sets of NAFLD patients with T2D according to the best identified cutoffs

	Se	Sp	PPV	NPV	Uncertainty area (%)
Advanced fibrosis—training set					
NFS					
Rule out <−1.539 (N = 119; 21.5%)	0.90 (0.86–0.94)	0.31 (0.26–0.36)	0.51 (0.46–0.55)	0.80 (0.71–0.87)	61.4
Rule in >0.766 (N = 95; 17.1%)	0.27 (0.21–0.33)	0.90 (0.86–0.93)	0.68 (0.58–0.78)	0.61 (0.56–0.65)	—
FIB4					
Rule out <0.973 (N = 294; 23.9%)	0.90 (0.87–0.92)	0.38 (0.34–0.42)	0.59 (0.56–0.62)	0.79 (0.74–0.83)	54.7
Rule in >2.310 (N = 264; 21.4%)	0.33 (0.29–0.37)	0.90 (0.87–0.92)	0.77 (0.71–0.82)	0.57 (0.54–0.60)	—
LSM					
Rule out <7.9 kPa (N = 390; 31.0%)	0.90 (0.87–0.92)	0.52 (0.48–0.56)	0.65 (0.62–0.68)	0.84 (0.80–0.88)	42.4
Rule in >14.6 kPa (N = 334; 26.6%)	0.44 (0.40–0.48)	0.90 (0.88–0.93)	0.82 (0.77–0.86)	0.62 (0.59–0.65)	—
AGILE 3+					
Rule out <0.426 (N = 338; 28.8%)	0.90 (0.87–0.92)	0.48 (0.44–0.52)	0.63 (0.60–0.67)	0.83 (0.78–0.86)	43.4
Rule in >0.848 (N = 327; 27.8%)	0.46 (0.42–0.50)	0.90 (0.87–0.92)	0.82 (0.78–0.86)	0.62 (0.59–0.66)	—
Advanced fibrosis—validation set					
NFS					
Rule out <−1.539 (N = 135; 30.2%)	0.91 (0.85–0.95)	0.20 (0.15–0.25)	0.40 (0.35–0.45)	0.79 (0.68–0.88)	57.9
Rule in >0.766 (N = 53; 11.9%)	0.25 (0.18–0.32)	0.92 (0.89–0.95)	0.65 (0.52–0.77)	0.68 (0.63–0.73)	—
FIB4					
Rule out <0.973 (N = 163; 36.3%)	0.88 (0.82–0.92)	0.41 (0.35–0.47)	0.46 (0.41–0.52)	0.85 (0.78–0.91)	45.2 —
Rule in >2.310 (N = 83; 18.5%)	0.24 (0.17–0.31)	0.95 (0.92–0.97)	0.74 (0.60–0.85)	0.68 (0.63–0.73)	—
LSM					
Rule out <7.9 kPa (N = 190; 43.6%)	0.81 (0.74–0.86)	0.46 (0.40–0.52)	0.47 (0.41–0.52)	0.80 (0.73–0.86)	37.4 —
Rule in >14.6 kPa (N = 83; 19.0%)	0.35 (0.28–0.43)	0.91 (0.87–0.94)	0.70 (0.59–0.79)	0.71 (0.66–0.75)	
AGILE3					
Rule out <0.426 (N = 191; 44.6%)	0.86 (0.79–0.91)	0.61 (0.54–0.66)	0.56 (0.49–0.62)	0.88 (0.82–0.92)	34.8
Rule in >0.848 (N = 88; 20.6%)	0.42 (0.35–0.51)	0.95 (0.91–0.97)	0.82 (0.72–0.90)	0.74 (0.69–0.78)	—
Fibrotic NASH—training set					
NFS					
Rule out <−1.539 (N = 73; 18.0%)	0.90 (0.85–0.93)	0.30 (0.23–0.37)	0.65 (0.59–0.70)	0.67 (0.55–0.78)	62.5
Rule in >0.674 (N = 79; 19.5%)	0.26 (0.21–0.32)	0.90 (0.85–0.94)	0.80 (0.69–0.88)	0.46 (0.41–0.52)	—
FIB4					
Rule out <0.845 (N = 106; 18.3%)	0.90 (0.86–0.93)	0.31 (0.25–0.38)	0.67 (0.63–0.71)	0.67 (0.57–0.76)	63.3
Rule in >2.306 (N = 107; 18.4%)	0.24 (0.20–0.29)	0.90 (0.86–0.94)	0.79 (0.71–0.87)	0.43 (0.39–0.48)	—

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TABLE 4. (*continued*)

	Se	Sp	PPV	NPV	Uncertainty area (%)
Advanced fibrosis—training set					
LSM					
Rule out <6.6 kPa (N = 117; 19.3%)	0.90 (0.86–0.93)	0.33 (0.27–0.39)	0.67 (0.62–0.71)	0.69 (0.60–0.77)	52.1
Rule in >14.0 kPa (N = 173; 28.5%)	0.41 (0.36–0.47)	0.90 (0.86–0.94)	0.87 (0.81–0.91)	0.51 (0.46–0.56)	—
AGILE3					
Rule out <0.288 (N = 101; 18.5%)	0.90 (0.86–0.93)	0.32 (0.26–0.39)	0.68 (0.63–0.72)	0.67 (0.57–0.76)	52.7
Rule in >0.819 (N = 157; 28.8%)	0.41 (0.36–0.47)	0.90 (0.86–0.94)	0.87 (0.81–0.92)	0.49 (0.44–0.54)	—
Fibrotic NASH—validation set					
NFS					
Rule out <−1.539 (N = 64; 16.2%)	0.80 (0.74–0.86)	0.42 (0.35–0.49)	0.58 (0.52–0.64)	0.68 (0.58–0.76)	69.6
Rule in >0.674 (N = 56; 14.2%)	0.13 (0.09–0.19)	0.89 (0.84–0.93)	0.55 (0.40–0.70)	0.50 (0.45–0.56)	—
FIB4					
Rule out <0.845 (N = 109; 27.6%)	0.79 (0.73–0.84)	0.34 (0.28–0.41)	0.55 (0.49–0.61)	0.61 (0.52–0.71)	55.9
Rule in >2.306 (N = 65; 16.5%)	0.22 (0.16–0.28)	0.89 (0.84–0.93)	0.66 (0.53–0.77)	0.53 (0.47–0.58)	—
LSM					
Rule out <6.6 kPa (N = 125; 32.6%)	0.83 (0.77–0.88)	0.48 (0.41–0.55)	0.61 (0.55–0.67)	0.74 (0.65–0.81)	45.3
Rule in >14.0 kPa (N = 85; 22.1%)	0.35 (0.29–0.43)	0.91 (0.86–0.95)	0.80 (0.70–0.88)	0.59 (0.53–0.64)	—
AGILE3					
Rule out <0.288 (N = 124; 32.9%)	0.78 (0.71–0.83)	0.43 (0.36–0.51)	0.58 (0.51–0.64)	0.66 (0.57–0.74)	46.2
Rule in >0.819 (N = 79; 20.9%)	0.32 (0.25–0.39)	0.90 (0.85–0.94)	0.76 (0.65–0.85)	0.57 (0.51–0.63)	—

Abbreviations: LSM, liver stiffness measurement; NFS, NAFLD fibrosis score.

In our study, we also identified the best rule in (90% specificity) and rule out (90% sensitivity) thresholds for advanced fibrosis to be applied in the setting of diabetic patients. The higher sensitivity and specificity of these new cutoffs were at the cost of a higher uncertainty area ranging from about 42% for LSM to about 61% for NFS. Consequently, at the moment, traditional cutoffs applied in the general NAFLD population should be recommended also in the diabetic setting.

Our study also observed that in patients with NAFLD and T2D, LSM and its related score AGILE 3 + had a significantly lower accuracy for the diagnosis of advanced fibrosis in obese patients compared with nonobese patients and in those with elevated ALT compared with their counterpart. This finding confirms what was already been reported in the overall NAFLD population.[37] When looking at BMI, our results can raise the doubt that the lower accuracy of LSM in obese patients could be due to the use of the M instead of the XL probe. In an attempt to solve this question, we confirmed that the accuracy of LSM in the subgroup of patients where it was measured by M probe in nonobese and XL probe in obese was higher in nonobese patients compared with obese patients. Further studies assessing skin-to-capsule distance could add insights into this topic. On the other side, evidence in NAFLD already demonstrated that high ALT levels affect the accuracy of LSM for fibrosis by overestimating liver damage.[37] When looking at NFS and FIB-4, we observed that these scores performed better in patients older than 35 years who were nonobese and had normal ALT values. These data have already been reported in the NAFLD population, and they can be explained by the fact that these variables—included in the scores—are associated between them and with advanced fibrosis, but are also present in the absence of advanced fibrosis, therefore sometimes lowering the accuracy of noninvasive scores.

The comparison of NAFLD population with T2D to that without showed that noninvasive scores have similar diagnostic accuracy for advanced fibrosis in terms of AUROCs, even if they have lower sensitivity —except for FIB-4—but higher specificity and lower

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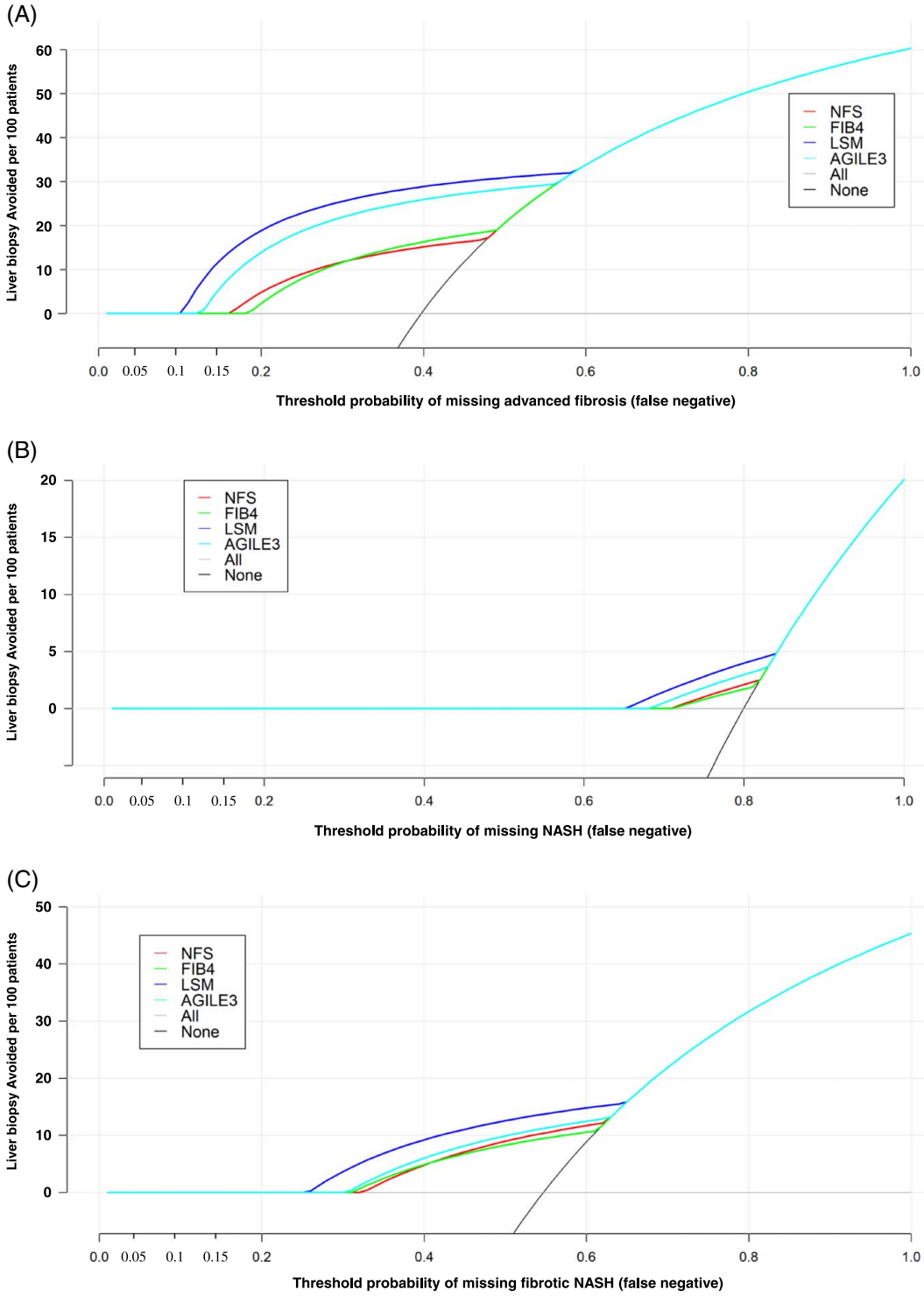

FIGURE 3 Net benefits by decision curve analyses of NFS, FIB-4, LSM, and AGILE 3+ for ruling out advanced liver fibrosis (A), NASH (B), and fibrotic NASH (C) by using new identified rule out cutoff in NAFLD patients with diabetes.

uncertainty area—except for LSM—in NAFLD without T2D. This trend was also confirmed when looking at sequential combination strategies, finally leading to a higher—even if modest—net benefit of noninvasive scores for ruling out advanced fibrosis in NAFLD patients without T2D. Notably, we also observed that 90% specificity rule in cutoff of LSM for advanced fibrosis was higher in NAFLD patients with T2D respect to those without (14.6vs. 11.8 kPa), this result being worthy to further validation clinical practice.

International regulatory agencies identified patients with fibrotic NASH as those eligible for clinical trials testing new pharmacological agents for NASH. In our study, we found that LSM and AGILE 3+, although

TABLE 5 Comparison in the entire cohort of diabetic NAFLD patients with T2D of AUCs of LSM and different scores for diagnosing F3-F4 fibrosis, F2-F4 fibrosis, NASH, and Fibrotic NASH according to obesity, age, and ALT levels

	Advanced fibrosis		NASH		Fibrotic NASH	
	AUC	*p*	AUC	*p*	AUC	*p*
BMI <30 kg/m^2 vs. BMI ≥30 kg/m^2						
APRI	0.75–0.66	0.034	0.69–0.50	0.004	0.74–0.71	0.531
NFS	0.80–0.70	0.030	0.71–0.59	0.061	0.77–0.73	0.531
FIB4	0.88–0.79	0.016	0.75–0.63	0.059	0.84–0.76	0.145
LSM	0.87–0.79	0.021	0.72–0.59	0.038	0.82–0.77	0.455
AGILE3	0.75–0.66	0.034	0.69–0.50	0.004	0.74–0.71	0.531
Normal ALT vs. abnormal ALT						
APRI	0.74–0.59	0.005	0.72–0.70	0.727	0.73–0.63	0.117
NFS	0.74–0.66	0.091	0.68–0.58	0.231	0.73–0.67	0.256
FIB4	0.80–0.68	0.011	0.72–0.64	0.308	0.78–0.72	0.256
LSM	0.87–0.82	0.091	0.80–0.70	0.231	0.86–0.76	0.028
AGILE3	0.88–0.79	0.015	0.78–0.65	0.153	0.84–0.77	0.139
Age <35 y vs. age 35–65 y						
APRI	0.47–0.72	0.125	0.45–0.71	0.208	0.67–0.73	0.981
NFS	0.54–0.74	0.166	0.83–0.66	0.570	0.51–0.73	0.245
FIB4	0.68–0.77	0.526	0.70–0.69	0.946	0.54–0.75	0.392
LSM	0.44–0.85	0.058	0.72–0.74	0.946	0.81–0.82	0.981
AGILE3	0.59–0.85	0.125	0.63–0.71	0.946	0.81–0.81	0.981
Age < 35 y vs. age > 65 y						
APRI	0.47–0.65	0.523	0.45–0.85	0.019	0.67–0.77	0.927
NFS	0.54–0.65	0.720	0.83–0.81	0.899	0.51–0.75	0.215
FIB4	0.68–0.69	0.948	0.70–0.83	0.893	0.54–0.77	0.338
LSM	0.44–0.84	0.073	0.72–0.85	0.899	0.81–0.85	0.954
AGILE3	0.59–0.82	0.346	0.63–0.84	0.624	0.81–0.82	0.954
Age 35–65 y vs. age > 65 y						
APRI	0.72–0.65	0.525	0.71–0.85	0.021	0.73–0.77	0.833
NFS	0.74–0.65	0.350	0.66–0.81	0.021	0.73–0.75	0.833
FIB4	0.77–0.69	0.350	0.69–0.83	0.021	0.75–0.77	0.833
LSM	0.85–0.84	0.798	0.74–0.85	0.021	0.82–0.85	0.833
AGILE3	0.85–0.82	0.798	0.71–0.84	0.021	0.81–0.82	0.833

Abbreviations: APRI, AST-to-platelet ratio indes; LSM, liver stiffness measurement; NFS, NAFLD fibrosis score.

originally developed or diagnosing liver fibrosis, overall outperformed NFS and FIB-4 and had acceptable accuracy for the diagnosis of fibrotic NASH. When looking at the best rule in and rule out cutoffs, the use of LSM and AGILE 3+ identified about 1 patient in three fourth at high risk of fibrotic NASH, keeping a specificity >90%. As for advanced fibrosis, in our DCA, only LSM showed a small benefit for ruling out fibrotic NASH at threshold probabilities of 5%, 10%, and 15% of missing fibrotic NASH, confirming the need for liver biopsy for correct identification of patients with fibrotic NASH, especially if the patient is eligible for inclusion in clinical trials. When comparing NAFLD population with T2D to those without, we found that the diagnostic accuracy of all noninvasive scores for fibrotic NASH was significantly lower in NAFLD patients with T2D. Notably, when looking at LSM, we also observed a lower uncertainty area and a higher rule in cutoff for fibrotic NASH in NAFLD patients with T2D with respect to those without (14 vs. 11.8 kPa). Different scores like NIS4[38] or MACK3[39] or cT1-AST-fasting glucose[40] have been recently proposed for the noninvasive identification of NAFLD patients with fibrotic NASH, but limited external validation and, most importantly, the use of not easily available unconventional variables limit their use in clinical practice. Along this line, magnetic resonance elastography (MRE)-based indices like MEFIB[41] and MAST[42] showed high PPV for fibrotic NASH, but the cost and availability of MRE limit their validation and their use in clinical practice. Otherwise, a simpler score, called FAST,[43] based on the combination of LSM, controlled attenuation parameter, and AST, has been

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recently shown to have good accuracy with an AUROC ranging from 0.74 to 0.85, a proportion of at high-risk patients ranging from 4% to 36%, and a specificity ranging from 82% to 99%. FAST could not be investigated in this IPDMA data set because controlled attenuation parameter was not available. Further studies in the setting of diabetic patients may demonstrate the superiority of FAST score—the today's standard for the diagnosis of fibrotic NASH—to LSM alone.

The main limitation of the present study lies in its potentially limited validity of the results in different populations and settings. As our study includes patients referred to tertiary hepatological referral centers for suspected liver damage, it is possible that the obtained results could not be replicated in general diabetic populations differing for age, biochemical alterations, the severity of T2D, and metabolic comorbidities. Along this line, the observed relatively high PPV and relatively low NPV of studied scores can be related to the high prevalence of advanced fibrosis, NASH, and fibrotic NASH in our population with respect to what is observed in the diabetic general population. Consequently, it could be possible that other cutoffs might be better in general diabetic populations with a lower prevalence of the investigated outcomes. The exclusion from IPDMA of studies using screening strategies other than LSM, and the hyporepresentation of North or South America populations, where T2D is highly prevalent, could further limit the generalizability of our results. The allowed 6-month interval between NITs and liver biopsy could also affect the interpretation of the results: this interval time could be considered substantial for NASH and fibrotic NASH where inflammation and steatosis components can significantly change within this timeframe. Moreover, the observed performance of NITs in subgroup analyses can be affected by the spectrum bias effect. Lack of an external validation cohort, central biopsy reading, and potentially hidden alcohol abuse in some patients could further limit the interpretation of our results.

In conclusion, we demonstrated that in the setting of NAFLD patients with T2D, LSM, and AGILE 3+ have similar good and acceptable diagnostic accuracy for the diagnosis of advanced fibrosis and fibrotic NASH, respectively. In the context of their limited availability, the sequential combination of serum-based with LSM-based tests for advanced fibrosis lead to a reduction of about 40%–60% in necessary LSM tests keeping sensitivity and specificity $\geq$80% for NFS-based and FIB-4–based combinations.

AUTHOR CONTRIBUTIONS

Ferenc E. Mózes: data curation, Michael Pavlides: data curation and individual patient data, Jeremy F.L. Cobbold: individual patient data. All authors contributed significant intellectual content and approved the final manuscript.

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CONFLICTS OF INTEREST

Guruprasad P. Aithal consults and advises through Nottingham University Consultants Team. Yusuf Yilmaz consults for Echosens. Jerome Boursier consults for Echosens. Victor de Ledinghen is on the speakers' bureau for Echosens. Wah Kheong Chan consults for or advises Abbvie, Boehringer Ingelheim, Novo Nordisk, and Roche and is on the speakers' bureau for Viatris and Hisky Medical. Thomas Karlas advises, is on the speakers' bureau for, and received grants from Echosens. Johannes Wiegand received grants from Echosens. Vincent Wai-Sun Wong consults and received grants from Gilead. He consults for AbbVie, Boehringer Ingelheim, Echosens, Intercept, Inventiva, Novo Nordisk, Pfizer, Sagimet Biosciences, and TARGET PharmaSolutions. Vincent Wai-Sun Wong consults for or advises Boehringer Ingelheim, Echosens, Intercept, Inventiva, Pfizer, Sagimet Biosciences, and TARGET PharmaSolutions. He consults for or advises, and is on the speakers' bureau for AbbVie and NovoNordisk. He is on the speakers' bureau for Abbott. He consults for, advises, and received grants from Gilead. He is a cofounder of Illuminatio Medical Technology Limited. Jorn Schattenberg consults for, is on the speakers' bureau for and received grants from Boehringer Ingelheim and Histoindex. He consults for and is on the speakers' bureau for Novo Nordisk, Madrigal, and Echosens. He consults for and received grants from Gilead. He consults for Apollo Endosurgery, Albireo, Bayer, BMS, GSK, Intercept Pharmaceuticals, Ipsen,

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Inventiva Pharma, MSD, Northsea Therapeutics, Novartis, Pfizer, Roche, Sanofi, and Siemens Healthineers. He is on the speakers' bureau for MedPublico GmbH. He received grants from Siemens Healthcare GmbH. Won Kim consults for and is on the speakers' bureau for Boehringer Ingelheim, Novonordisk, HK Inoen, Standigm, PharmaKing, KOBIOLABS, Ildong, Olix Pharma, Samil, TSD Life Sciences, Daewoong Pharmaceutical, and Eisai. He received grants from Gilead, Novartis, Pfizer, Roche, Ildong, Galmed, Dicerna, Celgene, and Enyo. He owns stocks in KOBIOLABS and Lepidyne. Katharina Staufer is an employee of Versantis AG. Michael Trauner advises, is on the speakers' bureau for, and received grants from Abbvie, Albireo, BIOMx, BI, Falk, Gilead, Genfit, Hightide, Intercept, Janssen, MSD, Novartis, Phenex, Pliant, Regulus, Siemens, Shire, and BMS. He received grants from Alnylam, Cymabay, Takeda, and UltraGenyx. He is listed as a coinventor on a patent filed by The Medical Universities of Graz and Vienna on medical use of norUDCA. Elisabetta Bugianesi consults for and advises Novo Nordisk. She consults for and is on the speakers' bureau for MSD. She is on the speakers' bureau for and received grants from Gilead. She consults for Boehringer Ingelheim, Lilly, and Novo Nordisk. Ramy Younes is employed by Boehringer Ingelheim. Ming-Hua Zheng is on the speakers' bureau for Hisky Medical. Calogero Camma advises Eisai, Ipsen, Roche, and AstraZeneca. Quentin M. Anstee consults for on behalf of Newcastle University, Alimentiv, Akero, AstraZeneca, Axcella, 89Bio, Boehringer Ingelheim, Bristol Myers Squibb, Galmed, Genfit, Genentech, Gilead, GlaxoSmithKline, Hanmi, HistoIndex, Intercept, Inventiva, Ionis, IQVIA, Janssen, Madrigal, Medpace, Merck, NGMBio, Novartis, Novo Nordisk, PathAI, Pfizer, Prosciento, Poxel, Resolution Therapeutics, Roche, Ridgeline Therapeutics, RTI, Shionogi, and Terns. He is on the speaker' bureau for Fishawack, Integritas Communications, Kenes, Novo Nordisk, Madrigal, Medscape, and Springer Healthcare. He received grants from AstraZeneca, Boehringer Ingelheim, and Intercept. He serves on the DSMB, on behalf of Newcastle University, for Medpace (North Sea Therapeutics). Michael Pavlides owns stock in Perspectum Ltd. Salvatore Petta advises and is on the speakers' bureau for AbbVie, Echosens, Gilead, Intercept, MSD, Novonordisk, and Pfizer. The remaining authors have no conflicts to report.

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REFERENCES

1. Le MH, Yeo YH, Li X, Li J, Zou B, Wu Y, et al. 2019 Global NAFLD Prevalence: a systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2021;20:2809-2817.e28.
2. Harris R, Harman DJ, Card TR, Aithal GP, Guha IN. Prevalence of clinically significant liver disease within the general population, as defined by non-invasive markers of liver fibrosis: a systematic review. Lancet Gastroenterol Hepatol. 2017;2:288–97.
3. Gu W, Hortlik H, Erasmus HP, Schaaf L, Zeleke Y, Uschner FE, et al. Trends and the course of liver cirrhosis and its complications in Germany: nationwide population-based study (2005 to 2018). Lancet Reg Health Eur. 2021;12:100240.
4. Vitale A, Svegliati-Baroni G, Ortolani A, Cucco M, Dalla Riva GV, Giannini EG, et al. Epidemiological trends and trajectories of MAFLD-associated hepatocellular carcinoma 2002-2033: the ITA.LI.CA database. Gut. 2021;72:141–52.
5. Taylor RS, Taylor RJ, Bayliss S, Hagström H, Nasr P, Schattenberg JM, et al. Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis. Gastroenterology. 2020;158:1611–25.e12.
6. Pennisi G, Enea M, Romero-Gomez M, Viganò M, Bugianesi E, Wong VWS, et al. Liver-related and extrahepatic events in patients with non-alcoholic fatty liver disease: a retrospective competing risks analysis. Aliment Pharmacol Ther. 2022;55:604–15.
7. Mantovani A, Petracca G, Beatrice G, Tilg H, Byrne CD, Targher G. Nonalcoholic fatty liver disease and risk of incident diabetes mellitus: an updated meta-analysis of 501 022 adult individuals. Gut. 2021;70:962–69.
8. Kanwal F, Kramer JR, Li L, Dai J, Natarajan Y, Yu X, et al. Effect of metabolic traits on the risk of cirrhosis and hepatocellular cancer in nonalcoholic fatty liver disease. Hepatology. 2020;71:808–19.
9. Younossi ZM, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: a systematic review and meta-analysis. J Hepatol. 2019;71:793–801.
10. Berzigotti A, Tsochatzis E, Boursier J, Castera L, Cazzagon N, Friedrich-Rust M, et al. European Association for the Study of the Liver. EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis - 2021 update. J Hepatol. 2021;75:659–89.
11. American Diabetes Association. 4. Comprehensive Medical Evaluation and Assessment of Comorbidities: Standards of Medical Care in Diabetes-2020. Diabetes Care. 2020;43:S37–47.
12. Mózes FE, Lee JA, Selvaraj EA, Jayaswal ANA, Trauner M, Boursier J, et al. Diagnostic accuracy of non-invasive tests for advanced fibrosis in patients with NAFLD: an individual patient data meta-analysis. Gut. 2022;71:1006–9.
13. Selvaraj EA, Mózes FE, Jayaswal ANA, Zafarmand MH, Vali Y, Lee JA, et al. Diagnostic accuracy of elastography and magnetic resonance imaging in patients with NAFLD: a systematic review and meta-analysis. J Hepatol. 2021;75:770–85.
14. Bril F, McPhaul MJ, Caulfield MP, Clark VC, Soldevilla-Pico C, Firpi-Morell RJ, et al. Performance of plasma biomarkers and diagnostic panels for nonalcoholic steatohepatitis and advanced fibrosis in patients with type 2 diabetes. Diabetes Care. 2020;43:290–7.
15. Boursier J, Canivet CM, Costentin C, Lannes A, Delamarre A, Sturm N, et al. Impact of type 2 diabetes on the accuracy of noninvasive tests of liver fibrosis with resulting clinical implications. Clin Gastroenterol Hepatol. 2022:S1542-3565(22)00248-00248.
16. Blank V, Petroff D, Beer S, Böhlig A, Heni M, Berg T, et al. Current NAFLD guidelines for risk stratification in diabetic patients have poor diagnostic discrimination. Sci Rep. 2020;10:18345.
17. Sanyal AJ, et al. Enhanced diagnosis of advanced fibrosis and cirrhosis in individuals with NAFLD using FibroScan-based Agile scores. J Hepatol. 2023;78:247–59.
18. Pennisi G, Enea M, Pandolfo A, Celsa C, Antonucci M, Ciccioli C, et al. AGILE 3+ Score for the diagnosis of advanced fibrosis and for predicting liver-related events in NAFLD. Clin Gastroenterol Hepatol. 2022:S1542-3565(22)00646-2.
19. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. Non-cirrhotic nonalcoholic steatohepatitis with liver fibrosis: developin drugs for treatment. Guidance for industry [draft guidance]. US Food and Drug Administration. 2018. Accessed June 14, 2021. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/noncirrhotic-nonalcoholicsteatohepatitis-liver-fibrosis-developing-drugs-treatment
20. Loomba R, Ratziu V, Harrison SA, Loomba R, McFarlane SC, Tamaki N, et al. Expert panel review to compare FDA and EMA guidance on drug development and endpoints in nonalcoholic steatohepatitis. Gastroenterology. 2022;162:680–8.
21. Okajima A, Sumida Y, Taketani H, Hara T, Seko Y, Ishiba H, et al. Liver stiffness measurement to platelet ratio index predicts the stage of liver fibrosis in non-alcoholic fatty liver disease. Hepatol Res. 2017;47:721–30.
22. Ooi GJ, Earnest A, Kemp WW, Burton PR, Laurie C, Majeed A, et al. Evaluating feasibility and accuracy of non-invasive tests for nonalcoholic fatty liver disease in severe and morbid obesity. Int J Obes. 2018;42:1900–1.
23. Seki K, Shima T, Oya H, Mitsumoto Y, Mizuno M, Okanoue T. Assessment of transient elastography in Japanese patients with non-alcoholic fatty liver disease. Hepatol Res. 2017;47:882–9.
24. Ziol M, Kettaneh A, Ganne-Carrié N, Barget N, Tengher-Barna I, Beaugrand M. Relationships between fibrosis amounts assessed by morphometry and liver stiffness measurements in chronic hepatitis or steatohepatitis. Eur J Gastroenterol Hepatol. 2009;21:1261–8.
25. Garg H, Aggarwal S, Shalimar, Yadav R, Datta Gupta S, Agarwal L, et al. Utility of transient elastography (fibroscan) and impact of bariatric surgery on nonalcoholic fatty liver disease (NAFLD) in morbidly obese patients. Surg Obes Relat Dis. 2018;14:81–91.
26. Introduction: Standards of Medical Care in Diabetes-2020. Diabetes Care. 2020;43 (suppl 1):S1–2.
27. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41:313–21.
28. McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut. 2010;59:1265–69.
29. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007;45:846–54.
30. Lin Z-H, Xin YN, Dong QJ, Wang Q, Jiang XJ, Zhan SH, et al. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. Hepatology. 2011;53:726–36.
31. Wong VWS, Vergniol J, Wong GLH, Foucher J, Chan HLY, Le Bail B, et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology. 2010;51:454–62.
32. Prati D, Taioli E, Zanella A, Torre ED, Butelli S, Del Vecchio E, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. Ann Intern Med. 2002;137:1–10.

33. Vickers AJ, Elkin EB. Decision curve analysis: a novel method for evaluating prediction models. Med Decis Making. 2006;26:565–74.
34. Vickers AJ, van Calster B, Steyerberg EW. A simple, step-by-step guide to interpreting decision curve analysis. Diagn Progn Res. 2019;3:18.
35. R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2021.https://www.R-project.org/
36. Zhang Z, Rousson V, Lee WC, Ferdynus C, Chen M, Qian X, et al. Decision curve analysis: a technical note. Ann Transl Med. 2018;6:308.
37. Petta S, Wai-Sun Wong V, Bugianesi E, Fracanzani AL, Cammà C, Hiriart JB, et al. Impact of obesity and alanine aminotransferase levels on the diagnostic accuracy for advanced liver fibrosis of noninvasive tools in patients with nonalcoholic fatty liver disease. Am J Gastroenterol. 2019;114:916–28.
38. Harrison SA, Ratziu V, Boursier J, Francque S, Bedossa P, Majd Z, et al. A blood-based biomarker panel (NIS4) for non-invasive diagnosis of non-alcoholic steatohepatitis and liver fibrosis: a prospective derivation and global validation study. Lancet Gastroenterol Hepatol. 2020;5:970–85.
39. Boursier J, Anty R, Vonghia L, Moal V, Vanwolleghem T, Canivet CM, et al. Screening for therapeutic trials and treatment indication in clinical practice: MACK-3, a new blood test for the diagnosis of fibrotic NASH. Aliment Pharmacol Ther. 2018;47:1387–96.
40. Dennis A, Mouchti S, Kelly M, Fallowfield JA, Hirschfield G, Pavlides M, et al. A composite biomarker using multiparametric magnetic resonance imaging and blood analytes accurately identifies patients with non-alcoholic steatohepatitis and significant fibrosis. Sci Rep. 2020;10:15308.
41. Jung J, Loomba RR, Imajo K, Madamba E, Gandhi S, Bettencourt R, et al. MRE combined with FIB-4 (MEFIB) index in detection of candidates for pharmacological treatment of NASH-related fibrosis. Gut. 2021;70:1946–53.
42. Noureddin M, Truong E, Gornbein JA, Saouaf R, Guindi M, Todo T, et al. MRI-based (MAST) score accurately identifies patients with NASH and significant fibrosis. J Hepatol. 2022;76:781–87.
43. Newsome PN, Sasso M, Deeks JJ, Paredes A, Boursier J, Chan WK, et al. FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study. Lancet Gastroenterol Hepatol. 2020;5:362–73.

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