The diagnostic utility of fibrosis-4 or nonalcoholic fatty liver disease fibrosis score combined with liver stiffness measurement by fibroscan in assessment of advanced liver fibrosis: a biopsy-proven nonalcoholic fatty liver disease study

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Background/aim: The clinical guidelines recommend the use of nonalcoholic fatty liver disease fibrosis score and fibrosis-4 score for estimating the advanced liver fibrosis in nonalcoholic fatty liver disease. However, these scores are used confidently in eliminating advanced fibrosis, rather than detecting it. Therefore, paired combination with liver stiffness measurement by transient elastography is recommended. In this study, we aimed to validate this combined algorithm in our study population.

Methods: A total of 139 consecutive biopsy-proven nonalcoholic fatty liver disease patients were enrolled in the study. We calculated the noninvasive scores and performed liver stiffness measurement examination for each patient.

Results: The optimal cutoff of liver stiffness measurement for advanced fibrosis was 11.0 kPa (area under curve: 0.856) with a sensitivity of 84% and a specificity of 78%. Using the fibrosis-4 score (< 1.45 for low risk of advanced fibrosis and > 3.25 for high risk of advanced fibrosis) in combination with the liver stiffness measurement cutoffs revealed the best diagnostic performance (< 8.8 kPa for low risk of advanced fibrosis and > 10.9 kPa for high risk of advanced fibrosis). This paired combination had the positive predictive value of 0.735 at a sensitivity of 89% and the negative predictive value of 0.932 at a specificity of 82%.

Conclusion: A paired combination of the fibrosis-4 score and liver stiffness measurement (< 8.8 kPa for exclusion of advanced fibrosis and > 10.9 kPa for inclusion of advanced fibrosis) is able to diagnose the patients with advanced fibrosis with the highest diagnostic accuracy. Eur J Gastroenterol Hepatol 2020: 642–649

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is estimated to have a worldwide prevalence of 25%, making it the most common etiology of the chronic liver diseases [1]. Histologically, NAFLD consists of a wide spectrum from hepatic steatosis without additional histological determinants of liver injury to nonalcoholic steatohepatitis (NASH) with or without fibrosis. However, this range of histological diagnosis does not indicate an inevitable progress from hepatic steatosis to NASH or from NASH to fibrosis, but NASH includes an increased risk of developing hepatic fibrosis, which is the most relevant histological term independently associated with liver related morbidity and mortality [2,3]. Indeed, advanced fibrosis is the leading factor for increased risk of mortality among NAFLD patients, which underlines the importance of subsequential referrals of appropriate patients [4], but an estimated advanced fibrosis prevalence of less than 5% among NAFLD patients indicates a need for first-line stratification strategy for an optimal management of the disease [5,6].

For staging of hepatic fibrosis, a liver biopsy is considered the reference standard. However, to perform a liver biopsy in each NAFLD patient for the staging of hepatic fibrosis is both unsuitable and limited considering its invasiveness, high cost, and patient discomfort [7,8]. Therefore, many noninvasive scoring systems and techniques were developed. Although the noninvasive tests mostly revealed to have similar diagnostic performances [9], the following modalities are recommended in the guidelines as the most accurate and validated ones [10,11]. For first-line stratification of advanced fibrosis in daily practice, the fibrosis-4 (FIB-4) score and the NAFLD Fibrosis Score (NFS), which classify patients as low risk, indeterminate risk, and high risk for advanced fibrosis, can be used confidently in primary care. On the other hand, magnetic resonance elastography and liver stiffness measurement (LSM) by transient elastography are more suitable for detecting advanced fibrosis by referral centers [12]. The paired combinations

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of these modalities have been shown to increase diagnostic accuracy. Therefore, their combined use can be recommended as reported previously [13,14]. In addition, recent reports have suggested that novel biomarkers such as a PRO-C3-based algorithm that included age, presence of diabetes, PRO-C3, and platelet count and DNA methylation marks could be employed as fibrosis biomarkers in NAFLD [15,16]. More recently, a tissue inhibitor of metalloproteinase-1 was recommended as a clinically useful marker for distinguishing NAFLD patients with and without significant fibrosis [17].

In this study, we primarily aimed to validate the use of NFS and FIB-4 score solitary and in combination with LSM in our study population. Secondarily, we aimed to investigate single and combined diagnostic performances of noninvasive tests to identify advanced fibrosis for our population.

Patients and methods

Patients

We enrolled a total of 139 biopsy-proven adult (≥ 18 years) NAFLD patients to our study. Demographics (age at the time of biopsy, gender), clinical data (height, weight, BMI, waist circumference, systolic and diastolic blood pressures) and laboratory (fasting blood glucose, liver function test, lipid profile) data were recorded. We excluded the patients with the following conditions: presence of any secondary cause for hepatic fat accumulation [e.g. significant alcohol consumption (> 30 g of alcohol per day for men and > 20 grams of alcohol per day for women) and previous history of steatogenic drugs use], presence of chronic liver diseases, significant weight loss or gain between liver biopsy and LSM examination. The maximum time interval between liver biopsy and LSM examination was six months for each patient.

Calculation of the nonalcoholic fatty liver disease fibrosis score and fibrosis-4 score

Using universally accepted formulas, we calculated the NFS as: $-1.675 + 0.037 \times$ age (years) $+ 0.094 \times$ BMI (kg/m^2) $+ 1.13 \times$ impaired fasting glucose/diabetes (yes = 1, no = 0) $+ 0.99 \times$ aspartate transaminase/alanine transaminase ratio $- 0.013 \times$ platelet ($\times 10^9$/L) $- 0.66 \times$ albumin (g/dl) [18] and the FIB-4 score as: age (years) $\times$ aspartate transaminase (U/L) / platelets (10^9/L) $\times \sqrt{}$alanine transaminase (U/L) [17]. For NFS, a score of < –1.455 indicated low risk for advanced fibrosis and > 0.676 high risk for advanced fibrosis [18]. Following the reported cutoffs for FIB-4 score, we separately used a score of < 1.3 [19] and < 1.45 [20] for low risk of advanced fibrosis and > 2.67 [19] and > 3.25 [20] for high risk of advanced fibrosis.

Performance of liver stiffness measurement by transient elastography

All of the LSM examinations were performed by a single operator using the FibroScan 502 Touch device (Echosens SA, Paris, France) after the patients had been fasting for a minimum 3 hours as per the manufacturer's instructions. All the examinations were started with an M probe. Prompted by the automatic probe selection tool displayed in real time, which is based on the skin to liver capsule distance, the probe was switched to XL. For the LSM examinations, the patients were placed in the dorsal decubitus position, and the transducer probe was positioned in the intercostal space of the right lobe of the liver. The reliable transient elastography measurement was defined as at least 10 valid measurements and having the interquartile-range-to-median ratio of ≤ 0.3 [21,22]. In the study, to identify advanced fibrosis, we used the cutoffs from the report from the Baveno VI Consensus Workshop [23]. A cutoff of < 10 kPa was used to rule out advanced fibrosis and a cutoff of > 15 kPa for possessing a high probability of advanced fibrosis [23]. Moreover, we also calculated the best cutoff value that indicated advanced fibrosis specifically for our study population. For the calculation of the cutoff indicating high risk of advanced fibrosis and low risk of advanced fibrosis, we followed the standard procedure performed previously [24,25]. The low risk of LSM cutoff was set as < 80% of the optimal cutoff value revealed in our statistical analysis. Any value above the cutoff indicating low risk and below the cutoff indicating high risk, in that case the optimal cutoff, was detected as indeterminate.

Combined use of nonalcoholic fatty liver disease fibrosis score and fibrosis-4 score with liver stiffness measurement by transient elastography

After investigating the diagnostic performances of NFS, FIB-4 score and LSM individually, we followed a two-step approach. For the combined use of NFS and FIB-4 score with LSM, we used the approach summarized in Fig. 1. Misclassification was defined as approaching a misdiagnosis following the single- or two-step approach. Disagreement between NFS or FIB-score with LSM was considered discordance.

Liver histology

Liver histology specimens were evaluated by an expert pathologist and scored according to the NASH Clinical Research Network classification [26] and steatosis, activity, and fibrosis / fatty liver inhibition of progression algorithm [27]. The fibrosis stages (F) were classified as significant fibrosis (F ≥ 2) and advanced fibrosis (F ≥ 3), respectively.

Statistical analysis

Descriptive statistics were used to describe the general characteristics of the study population. Continuous data were stated as mean, SD, median, and minimum–maximum; and the distribution normality of the variables was calculated with the Kolmogorov–Smirnov or the Shapiro–Wilk test. Categorical data were expressed as counts and proportions. Receiver operating characteristic (ROC) curve analysis was carried out to calculate the 95% confidence intervals (CIs) of the area under the curve (AUC) to identify the best cutoff point for LSM. The highest value for Youden's index was accepted as the best cutoff value. Youden's index was obtained from the coordinates of the curve and was calculated using the following formula: J = max [SN+SP] – 1. The diagnostic performances of the cutoff values including sensitivity, specificity, positive predictive value, and negative predictive value (NPV) were also presented. Cohen's Kappa value was

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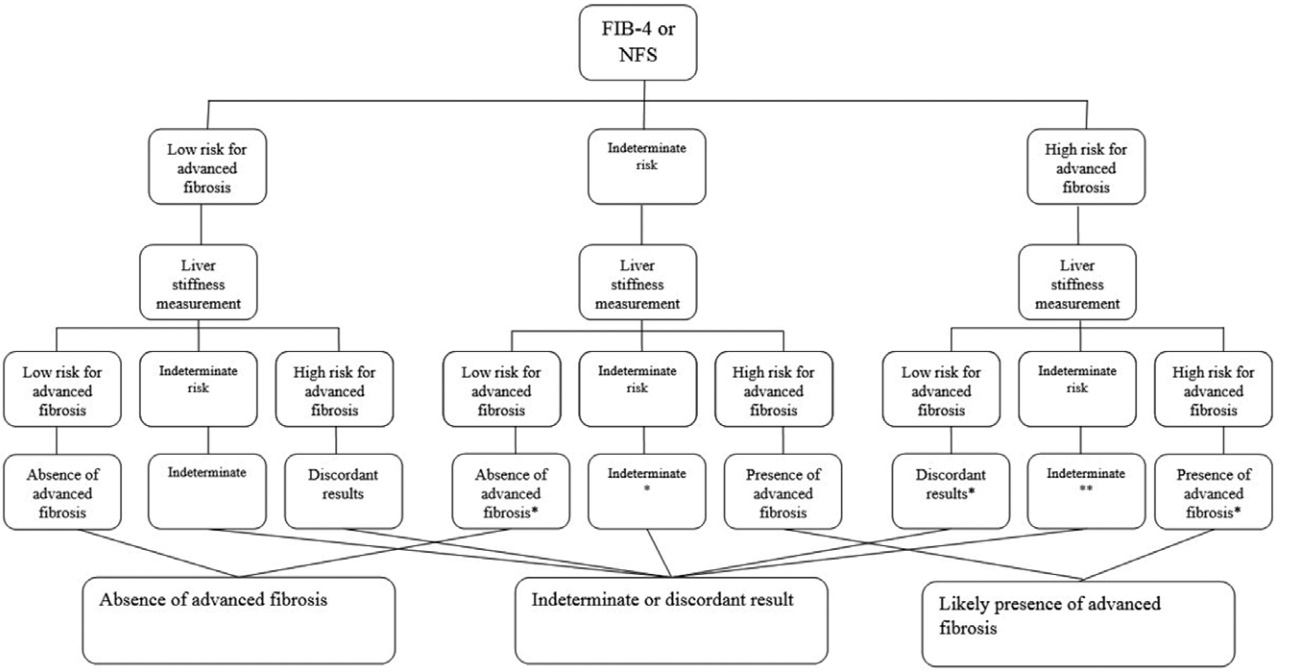


Fig. 1. Diagnostic approach of the use of combined modalities. FIB-4, fibrosis-4 score; NFS, nonalcoholic fatty liver disease fibrosis score.

Table 1. General characteristics and laboratory results of the study patients (n = 139)

Characteristics	
Age, mean ± SD, year	49 ± 12
Gender, male / female, n (%)[a]	82 (59) / 57 (41)[a]
BMI, mean ± SD, kg/m^2	32.9±11.5
Lean / overweight / obese, n (%)	5 (3.6) / 37 (26.6) / 97 (69.8)[a]
Metabolic syndrome, n (%)	110 (79.1)[a]
Type 2 diabetes mellitus, n (%)	89 (64)[a]
Hypertension, n (%)	77 (55.4)[a]
Waist circumference, mean ± SD, cm	107 ± 11
Albumin, median [minimum–maximum], mg/dl	4.6 [2.4–6.5]
AST, median [minimum–maximum], U/l	40 [15–174]
ALT, median [minimum–maximum], U/l	57 [13–288]
GGT, median [minimum–maximum], U/l	49 [13–505]
Total cholesterol, mean ± SD, mg/dl	205 ± 46
Triglycerides, median [minimum–maximum], mg/dl	167 [19–472]
HDL cholesterol, median [minimum–maximum], mg/dl	44 [19–140]
LDL cholesterol, median [minimum–maximum], mg/dl	129 [35–400]
Platelets, mean ± SD, ×10^3/µl	227 ± 59
Glucose, median [minimum–maximum], mg/dl	110 [70–307]
HbA1c, median [minimum–maximum], %	5.9 [4.5–11.1]
HOMA-IR	4.72 [1–28.76]
HOMA-IR > 2.7, n (%)	118 (84.9)[a]

ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; GGT, gamma-glutamyltransferase; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein.
[a]$P < 0.05$ was accepted as statistically significant.

accepted as a measurement of agreement with the reference standard.

Statistical analysis was performed using the IBM SPSS v.24 for Windows software and was reported with 95% CIs. $P < 0.05$ was accepted as statistically significant.

Ethics

The Turkish NAFLD biobank electronic database of the Institute of Gastroenterology at Marmara University was used in this study. This work was supported by the Scientific Research Projects Coordination Unit at Marmara University under grant number: SAG-C-TUP-150218-0033. The study was approved by the Ethics Committee of the Faculty of Medicine at Marmara University and performed in adherence to the principles of the Declaration of Helsinki.

Results

Characteristics of the study population

The study population consisted of 139 consecutive biopsy-proven NAFLD patients (age: 49 ± 12 years, 58% male). Of these, 97 (69.8%) were obese, 110 (79.1%) had metabolic syndrome, 89 (64%) had type 2 diabetes mellitus, and 77 (55.4%) had hypertension. The general characteristics of the patients are presented in Table 1. Histologically, a total of 49 patients (35.3%) were diagnosed with advanced fibrosis. Characteristics of the patients regarding transient elastography results, noninvasive scores, and histological examination are summarized in Table 2. According to the ROC analysis performed to detect the best cutoff point for LSM indicating significant fibrosis (F ≥ 2) and advanced fibrosis (F ≥ 3) for our study population, 8.95 kPa (AUC: 0.718, CI: 0.630–0.805) and 11.0 kPa (AUC: 0.856, CI: 0.792–0.920) were detected as best cutoff values, respectively. The single diagnostic performances of LSM examinations with those cutoffs are shown in Table 3.

Diagnostic performances of noninvasive tests in single and combined use with liver stiffness measurement

The diagnostic performances of noninvasive tests in individual use and combined with LSM in comparison to each other are presented in Table 3. According to our results, the FIB-4 (< 1.45, > 3.25) score, FIB-4 (< 1.3, > 2.67) score, and NFS (–1.455, > 0.676) had NPVs of 0.756, 0.808, and 0.833 at specificities of 94%, 91%, and 88%, respectively. On the other hand, the sensitivities of these tests were low to detect patients with advanced fibrosis (4%, 18%, and

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Table 2. Transient elastography results, noninvasive scores, and histological characteristics of the patients (n = 139)

Transient elastography	
Time between LSM examination and biopsy, median [minimum–maximum], day	33 [0–172]
Probe type (M/XL), n (%)	95 (68.3) / 44 (31.7)[a]
Number of measurements, median [minimum–maximum]	10 [10–14]
Reliable measurement	100%
Liver stiffness measurement, median [minimum–maximum], kPa	10.3 [4.1–72.0]
Liver stiffness measurement IQR, median [minimum–maximum]	1.6 [0.1–15.5]
IQR/M, median [minimum–maximum][a]	15 [0.1–29][a]
Controlled attenuation parameter, mean ± SD, dB/m	326.5 ± 41.5
Controlled attenuation parameter IQR, median [minimum–maximum]	25 [0–79]
Noninvasive scores (FIB-4 and NFS)	
FIB-4 score, median [minimum–maximum]	1.22 [0.35–4.50]
FIB-4 score low / indeterminate / high risk (< 1.3, > 2.67), n (%)	73 (52.5) / 57 (41) / 9 (6.5)[a]
FIB-4 score low / indeterminate / high risk (< 1.45, > 3.25), n (%)	90 (64.7) / 44 (31.7) / 5 (3.6)[a]
NFS, mean ± SD	−1.09 ± 1.29
NFS low / indeterminate / high risk (< −1.455, > 0.676), n (%)	54 (38.8) / 75 (54) / 10 (7.2)[a]
Histological characteristics	
SAF algorithm classification, NASH / NAFL, n (%)	137 (98.6) / 2 (1.4)[a]
Stage of activity (A) according to the SAF score: A1 / A2/ A3 / A4, n (%)	3 (2.2) / 38 (27.3) / 72 (51.8) / 26 (18.7)[a]
Stage of fibrosis (F) according to the SAF score: F0 / F1/ F2 / F3 / F4, n (%)	11 (7.9) / 40 (28.8) /39 (28.1) / 39 (28.1) / 10 (7.2)[a]
NAS score (NASH CRN), median [minimum–maximum]	6 [3-8]
NAS, < 3/ 3–4/ > 4, n (%)	0 (0) / 33 (23.7) / 106 (76.3)[a]
Significant fibrosis (≥ F2)/advanced fibrosis (≥ F3)/Cirrhosis (F4), n (%)	88 (63.3) / 49 (35.3) / 10 (7.2)[a]

CRN, Clinical Research Network; FIB-4, fibrosis-4 score; IQR, interquartile range; IQRM, median interquartile range; LSM, liver stiffness measurement; NAFL, nonalcoholic fatty liver; NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis; NFS, nonalcoholic fatty liver disease fibrosis score; SAF, steatosis, activity, and fibrosis.
[a]$P < 0.05$ was accepted as statistically significant.

Table 3. The diagnostic performances of liver stiffness measurement cutoffs for significant and advanced fibrosis in single use

	LSM ≥ 8.95 kPa indicating significant fibrosis (F ≥ 2)	LSM ≥ 11.0 kPa indicating advanced fibrosis (F ≥ 3)
Sensitivity (%)	76	84
Specificity (%)	59	78
Positive predictive value	0.761	0.672
Negative predictive value	0.588	0.897
Area under curve	0.718	0.856
Confidence interval	0.630–0.805	0.792–0.920
Kappa	0.350	0.582

LSM, liver stiffness measurement.
[a]$P < 0.05$ was accepted as statistically significant.

31%, respectively). Despite the suboptimal Kappa value (κ = 0.330), the FIB-4 (< 1.3, > 2.67) score had the highest agreement compared to the other noninvasive diagnostic tests applied to our population.

From all the paired combinations in the entire cohort, the best agreement was the paired combination of FIB-4 (< 1.45, > 3.25) score with LSM (< 8.8, 8.8–10.9, > 10.9 kPa) (κ = 0.681). On the other hand, this agreement was developed at a price of a major number of patients with discordant results (n=28, 20.1%; Table 4). Although the diagnostic performance of the FIB-4 score was generally good, a non-negligible percentage of the patients (44 of 139, 31.7%) fell into the gray area (indeterminate risk) for identifying advanced fibrosis in patients with NAFLD. More importantly, most of the patients (26 of 44, 59.1%) at indeterminate risk of advanced fibrosis based on FIB-4 score had advanced fibrosis on liver histology.

Distribution of the patients according to nonalcoholic fatty liver disease fibrosis score and fibrosis-4 score with combination of liver stiffness measurement

The distribution of the patients with and without advanced fibrosis according to the classification of noninvasive tests are presented in Fig. 2 and the recommended algorithm is shown in Fig. 3.

Fibrosis-4 (< 1.45, > 3.25) + liver stiffness measurement (< 8.8, 8.8–10.9, > 10.9 kPa) and fibrosis-4 (< 1.45, > 3.25) + liver stiffness measurement (< 10, 10–15, > 15 kPa)

Following the above algorithm for FIB-4 (< 1.45, > 3.25) score, a total of 90 patients were counted as low risk for advanced fibrosis, 44 patients as indeterminate and five patients as high risk for advanced fibrosis. From those, 22 patients (24.4%), 26 patients (59.1%) and one patient (20%) had advanced fibrosis in liver biopsy, respectively.

Since we performed the LSM examination following the cutoffs of < 8.8 kPa for low risk of advanced fibrosis and > 10.9 kPa for high risk of advanced fibrosis, we obtained the following results. From the 22 patients classified as low risk of advanced fibrosis wrongly, we were able to diagnose 16 patients correctly as having advanced fibrosis. On the other hand, following this algorithm, we overdiagnosed 11 patients as advanced fibrosis, who were correctly classified as low risk of advanced fibrosis according to the FIB-4s score. In the indeterminate zone, 22 of the 44 patients were diagnosed correctly as advanced fibrosis and seven patients were overdiagnosed as advanced fibrosis. From the five patients classified as high risk for advanced fibrosis, only one patient was diagnosed as advanced fibrosis after LSM.

With the performance of LSM with the cutoffs recommended at the Baveno VI Consensus Workshop, from the 22 patients classified as low risk of advanced fibrosis, we were able to detect seven patients correctly as advanced fibrosis and overdiagnosed two patients as advanced fibrosis. In the indeterminate zone, 10 patients were diagnosed correctly as advanced fibrosis and three patients were overdiagnosed as advanced fibrosis. From the five patients classified as high risk for advanced fibrosis, one patient was diagnosed correctly according to the LSM.

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Table 4. Comparison of the diagnostic performances of the noninvasive tests in single and paired combinations (n = 139)

	FIB-4[a]	FIB-4[a] + LSM[b]	FIB-4[a] + LSM[c]	FIB-4[d]	FIB-4[d] + LSM[b]	FIB-4[d] + LSM[c]	NFS	NFS + LSM[b]	NFS + LSM[c]
Sensitivity (%)	4	89	69	18	91	72	31	92	75
Specificity (%)	94	82	94	91	79	94	88	73	92
PPV	0.200	0.735	0.733	0.333	0.744	0.765	0.400	0.688	0.750
NPV	0.756	0.932	0.922	0.808	0.932	0.921	0.833	0.932	0.921
Misclassification, n (%)[e]	26 (18.7)	14 (10.1)	9 (6.5)	20 (14.4)	14 (10.1)	9 (6.5)	15 (10.8)	20 (14.4)	10 (7.2)
Indeterminate, n (%)[e]	44 (32)	33 (23.7)	48 (34.5)	57 (41)	33 (23.7)	48 (34.5)	75 (54)	33 (23.7)	48 (34.5)
Discordance, n (%)[e]	-	28 (20.1)	12 (8.6)	-	19 (13.7)	11 (7.9)	-	14 (10.1)	8 (5.8)
Kappa	0.310	0.681	0.639	0.330	0.677	0.671	0.253	0.612	0.671

FIB-4, fibrosis-4 score; LSM, liver stiffness measurement; NFS, nonalcoholic fatty liver disease fibrosis score; NPV, negative predictive value; PPV, positive predictive value.
[a]1.45 low risk for advanced fibrosis, > 3.25 high risk for advanced fibrosis.
[b]< 8.8 kPa for low risk of advanced fibrosis, 8.8–10.9 kPa for indeterminate risk, > 10.9 kPa for high risk of advanced fibrosis.
[c]< 10 kPa for low risk of advanced fibrosis, 10–15 kPa for indeterminate risk, > 15 kPa for high risk of advanced fibrosis.
[d]< 1.3 low risk for advanced fibrosis, > 2.67 high risk for advanced fibrosis.
[a]*P* < 0.05 was accepted as statistically significant.

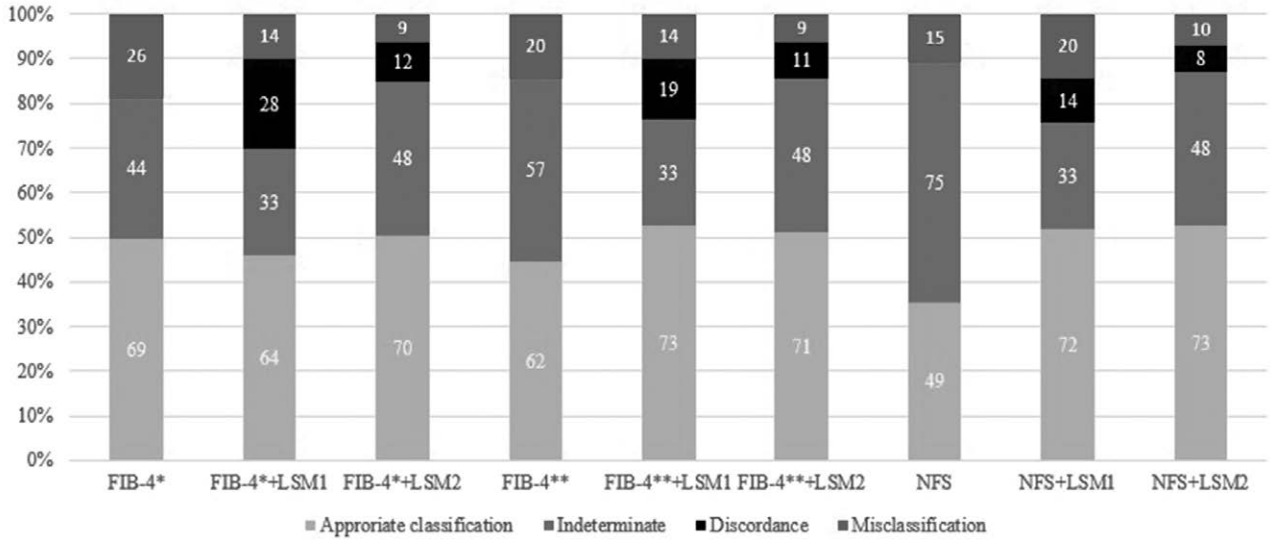


Fig. 2. The distributions according to the classification by noninvasive tests following the algorithm in Fig. 1 (n = 139). FIB-4, fibrosis-4 score; LSM, liver stiffness measurement; NFS, nonalcoholic fatty liver disease fibrosis score. *1.45 low risk for advanced fibrosis, > 3.25 high risk for advanced fibrosis; †< 1.3 low risk for advanced fibrosis, > 2.67 high risk for advanced fibrosis; a< 8.8 kPa for low risk of advanced fibrosis, 8.8–10.9 kPa for indeterminate risk, > 10.9 kPa for high risk of advanced fibrosis; b< 10 kPa for low risk of advanced fibrosis, 10–15 kPa for indeterminate risk, > 15 kPa for high risk of advanced fibrosis.

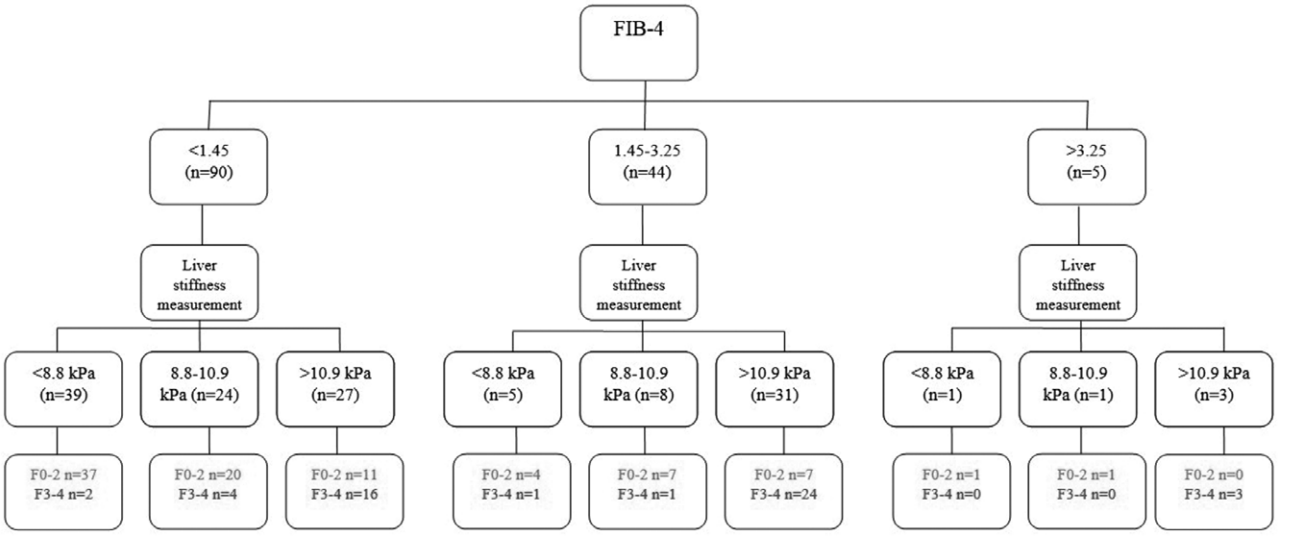


Fig. 3. The distribution of patients with advanced and non-advanced fibrosis according to the recommended algorithm (n = 139). FIB-4, fibrosis-4 score.

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Fibrosis-4 (< 1.3, > 2.67) + liver stiffness measurement (< 8.8, 8.8–10.9, > 10.9 kPa), fibrosis-4 (< 1.3, > 2.67) + liver stiffness measurement (< 10, 10–15, > 15 kPa)

According to the FIB-4 (< 1.3, > 2.67) score, we detected a total of 73 patients with low risk for advanced fibrosis, 57 patients as indeterminate and nine patients with high risk for advanced fibrosis. As for the liver histology, 14 out of 73 patients (19.2%) were detected in the low risk zone, 32 from 57 patients (56.1%) in the indeterminate zone, and 3 from 9 patients (33.3%) in the high risk for advanced fibrosis.

When we performed the LSM with the cutoff of < 8.8 kPa for low risk of advanced fibrosis and > 10.9 kPa for high risk of advanced fibrosis, from the 14 patients with advanced fibrosis in low risk of advanced fibrosis, we were able to detect nine correctly as advanced fibrosis. On the other hand, nine patients without advanced fibrosis in low risk of advanced fibrosis according to the FIB-4 score were overdiagnosed as advanced fibrosis. In the indeterminate zone, we were able to detect nearly all of the patients with advanced fibrosis (n = 31) according to the LSM results, overdiagnosing only seven as advanced fibrosis. From the nine patients with high risk of advanced fibrosis according to the FIB-4 score, three patients with advanced fibrosis were classified as advanced fibrosis and one patient as no advanced fibrosis, correctly.

Performing the LSM with the cutoffs recommended by Baveno VI Consensus Workshop, from the nine patients with advanced fibrosis in low risk of advanced fibrosis, six were able to be diagnosed, albeit two patients were overdiagnosed as advanced fibrosis. In the indeterminate zone, 11 of the 32 patients with advanced fibrosis were able to be diagnosed correctly as advanced fibrosis, whereas three patients without advanced fibrosis were classified as advanced fibrosis. From the nine patients with high risk of advanced fibrosis according to the FIB-4 score, three were diagnosed as advanced fibrosis and three as no advanced fibrosis, correctly.

Nonalcoholic fatty liver disease fibrosis score (< –1.455, > 0.676) + liver stiffness measurement (< 8.8, 8.8–10.9, > 10.9 kPa) and nonalcoholic fatty liver disease fibrosis score (< –1.455,> 0.676) + liver stiffness measurement (< 10, 10–15, > 15 kPa)

According to the NFS, we classified 54 patients as low risk for advanced fibrosis, 75 as indeterminate and 10 as high risk for advanced fibrosis. From those, nine patients (16.7%), 36 patients (48%), and four patients (40%) had advanced fibrosis according to the liver histology, respectively.

Using the cutoffs of < 8.8 kPa for low risk of advanced fibrosis and > 10.9 kPa for high risk of advanced fibrosis, from the nine patients in low risk for advanced fibrosis, according to the NFS, eight were able to be diagnosed correctly as advanced fibrosis whereas five patients were overdiagnosed. from the 36 patients in the indeterminate zone, 29 with advanced fibrosis were able to be diagnosed correctly. However, 12 patients in the indeterminate zone without advanced fibrosis were overdiagnosed. From the 10 patients, the results of NFS in four were supported in LSM and a correct diagnosis was made. One patient without advanced fibrosis was able to be classified correctly according to LSM.

When we used the cutoffs from the Baveno VI Consensus Workshop, from the nine patients with advanced fibrosis in low risk of advanced fibrosis zone according to NFS, four were diagnosed as advanced fibrosis and only one patient was overdiagnosed. In the indeterminate zone, from 36 patients with advanced fibrosis, 12 were diagnosed correctly as advanced fibrosis after LSM examination, and four were overdiagnosed. From the patients with high risk of advanced fibrosis according to NFS, in LSM examination, an estimation of three patients were supported correctly as advanced fibrosis by LSM, and three were opposed by LSM as without advanced fibrosis, correctly.

Discussion

In this study, we validated the two-step approach in detecting advanced fibrosis by noninvasive diagnostic tests in our study cohort. We investigated the diagnostic performances of each approach in our population and found that the best approach was the FIB-4 that accepted < 1.45 as low risk for advanced fibrosis and > 3.25 as high risk of advanced fibrosis, followed by LSM (< 8.8, 8.8–10.9, > 10.9 kPa) considering their highest Kappa values (κ = 0.681). With this algorithm, we provided also a decrease in the misclassification rate compared to the performance of FIB-4 alone. By performing the LSM after the FIB-4, the misclassification rate decreased from 18.7 to 10.1%. Moreover, we found that for both the NFS and FIB-4 score, approximately half the patients in the indeterminate zone had advanced fibrosis. For the patients taking the LSM cutoff < 8.8 for low risk of advanced fibrosis and > 10.9 for high risk of advanced fibrosis, which we detected as the best cutoff value for showing advanced fibrosis specifically in our study group, the LSM was able to detect nearly all the patients with advanced fibrosis in the indeterminate risk zone. Furthermore, we were able to determine LSM cutoffs for significant fibrosis and advanced fibrosis for our study population.

It was reported previously that in head-to-head comparisons, the FIB-4 score had a slightly better diagnostic performance than the NFS. Additionally, the FIB-4 score consisted of fewer parameters. Therefore, its calculation is simpler, and the method is more appropriate in triaging patients in primary care settings [5]. In our study, we also found that the use of FIB-4 score with the cutoffs < 1.45 for low risk of advanced fibrosis and > 3.25 for high risk of advanced fibrosis followed by the LSM cutoffs revealed relatively higher agreement with the reference standard in the ROC analysis compared to the other combinations.

A combined use of the NFS and FIB-4 score with LSM was previously recommended. According to that study, in combined use, the diagnostic accuracy increased, whereas the misclassification rate decreased in the indeterminate zone for advanced fibrosis [14]. Similarly, in our study, we observed that generally the misclassification rate decreased and we were able to detect the majority of patients with advanced fibrosis in the indeterminate zone. Moreover, in another study, the use of Baveno VI cutoff values for LSM following NFS or FIB-4 to reach the highest diagnostic accuracy was recommended [13].

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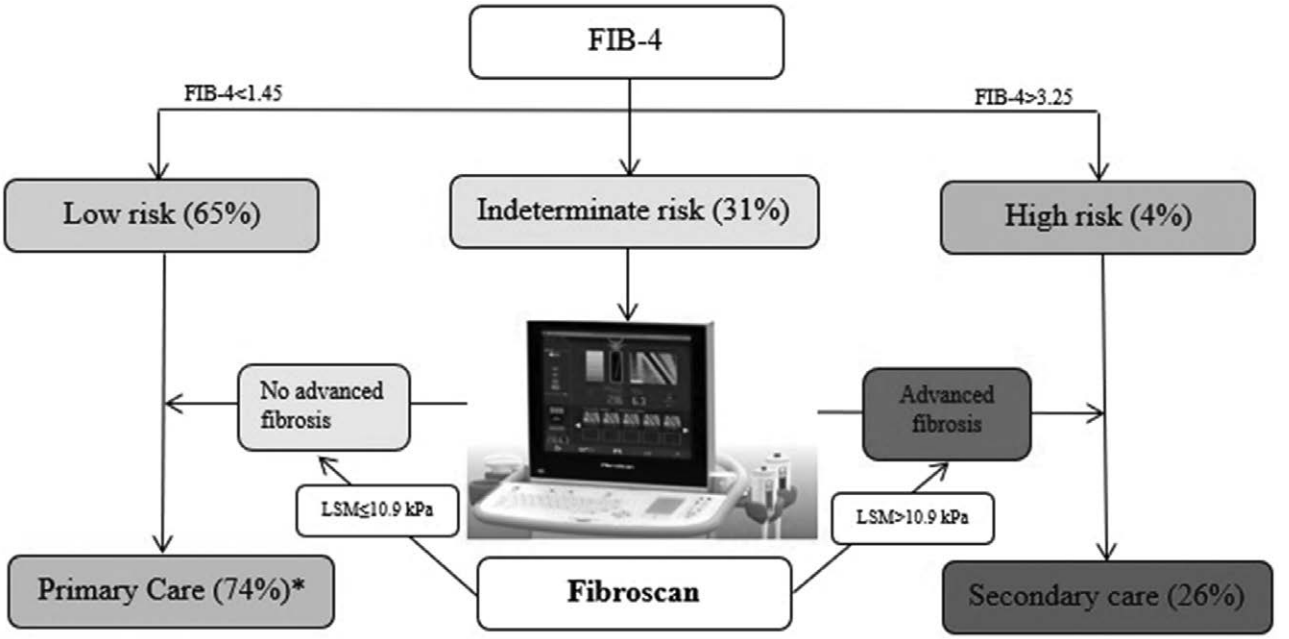


Fig. 4. Stepwise approach of screening for advanced fibrosis in primary care according to the recommended algorithm (n = 139). FIB-4, fibrosis-4 score; LSM, liver stiffness measurement. *Rescreen in three years.

As recommended in the European Association for the Study of the Liver Guidelines, the NFS, FIB-4 score, and LSM were the most validated diagnostic tools in the management of NAFLD, and even though their combined use was suggested, there was no consensus on the cutoffs for clinical practice [7]. The current study was compatible with the recommendation from the LSM cutoff point of view. Taking the cutoffs for FIB-4 as < 1.45 for low risk of advanced fibrosis and > 3.25 for high risk of advanced fibrosis, and for LSM < 8.8 kPa for low risk of advanced fibrosis and > 10.9 kPa for high risk of advanced fibrosis, we validated the recent approach tertiary care settings, which recommends the secondary care referral to patients classified as high risk for advanced fibrosis or revealed as advanced fibrosis in the fibroscan examination [6]. However, this high diagnostic accuracy was reached at a price of the presence of an uncertainty area and discordance which endorsed a secondary care risk stratification strategy without an indeterminate zone. On the other hand, for patients with low risk of advanced fibrosis according to the NFS and FIB-4 score, the diagnostic performance of NFS and FIB-4 score in single use for first-line stratification of advanced fibrosis was adequate. Therefore, we believe that patients classified as low risk of advanced fibrosis based on the FIB-4 score should be managed in primary care, whereas patients at high risk should be directed to secondary care. Patients at indeterminate risk should undergo Fibroscan examination (Fig. 4).

The strength of this study was based on the facts that the data were obtained from an experienced single-center with a total of more than 15 000 measurements by a single operator and that the evaluation of liver histology specimens were performed by a single pathologist. Moreover, we also produced LSM cutoffs indicating significant fibrosis, advanced fibrosis and cirrhosis specifically in a population of Turkish patients. On the other hand, we had several limitations to our study. First, as we started to use LSM, we avoided liver biopsies more frequently, which may have caused an underestimation of the number of patients without advanced fibrosis and led to a selection bias. Second, as our clinic is located in a tertiary care center, our study population may not reflect the general population with NAFLD [28]. Finally, due to the low number of patients, a definite differentiation between advanced fibrosis and cirrhosis could not be established.

In conclusion, we validated the two-step approach algorithm with NFS and FIB-4 score followed by LSM and we recommended to refer patients who were classified as indeterminate risk for advanced fibrosis to secondary care centers for further LSM examination. All in all, noninvasive scores such as the NFS and FIB-4 are useful tools for the exclusion of advanced fibrosis rather than its identification [29]. For the patients who were classified as indeterminate risk, we highly recommend Fibroscan examination. According to our study, a combined use of the FIB-4 (< 1.45 for low risk of advanced fibrosis, > 3.25 for high risk of advanced fibrosis) score with LSM (< 8.8 kPa for exclusion of advanced fibrosis, > 10.9 kPa for inclusion of advanced fibrosis) was able to diagnose the patients with advanced fibrosis with highest diagnostic accuracy in our study population (sensitivity: 89%, specificity: 82%, positive predictive value: 0.735, NPV: 0.932). Following that approach, it would be possible to avoid liver biopsy and to decrease cost and complications.

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Conflicts of interest

There are no conflicts of interest.

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