ORIGINAL ARTICLE

Gut and Liver, Vol. 14, No. 4, July 2020, pp. 486-491

Simple Noninvasive Scores Are Clinically Useful to Exclude, Not Predict, Advanced Fibrosis: A Study in Turkish Patients with Biopsy-Proven Nonalcoholic Fatty Liver Disease

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Background/Aims: Advanced fibrosis (F≥3) indicates poor outcomes in nonalcoholic fatty liver disease (NAFLD). Here, we examined the diagnostic performance of the fibrosis-4 index (FIB-4) and NAFLD fibrosis score (NFS) for detecting (or excluding) advanced fibrosis in patients with biopsy-proven NAFLD. **Methods:** The diagnostic performance of each noninvasive test according to previously identified cutoff points indicating low and high risk for advanced fibrosis was determined in 463 patients with NAFLD. Patients who scored <1.3 and >2.67 on the FIB-4 were considered at low and high risk for advanced fibrosis, respectively. Patients who scored <−1.455 and >0.676 on the NFS were considered at low and high risk for advanced fibrosis, respectively. **Results:** Eighty-one patients (17.5%) had biopsy-proven advanced fibrosis (F≥3). The published FIB-4 cutoff values for low and high risk were able to exclude advanced fibrosis with negative predictive values (NPVs) of 0.907 and 0.843 and specificities of 74% and 97%, respectively. The published NFS cutoff values for low and high risk were able to exclude advanced fibrosis with NPVs of 0.913 and 0.842 and specificities of 63% and 96%, respectively. If biopsies were performed in only patients with a FIB-4 above the low cutoff point (≥1.3), 67.1% could be avoided. Conversely, if biopsies were performed in only patients with an NFS above the low cutoff point (≥−1.455), 57.0% could be avoided. **Conclusions:** The main clinical utility of the FIB-4 and NFS in patients with NAFLD lies in the ability to exclude, not identify, advanced fibrosis. **(Gut Liver 2020;14:486-491)**

Key Words: Non-alcoholic fatty liver disease; Liver fibrosis; Diagnostic test; Sensitivity and specificity

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD)–the hepatic manifestation of the metabolic syndrome–is a growing public health concern and the most common cause of chronic liver diseases worldwide, with an estimated overall prevalence of 25%.[1] Accumulating evidence indicates that the severity of hepatic fibrosis is the main prognostic determinant in NAFLD.[2] Accordingly, patients with advanced fibrosis (F≥3) are more likely to experience hepatic complications and have higher liver-related, cardiovascular, and overall mortality rates.[2,3] In this scenario, a timely detection of advanced fibrosis is clinically paramount for prioritizing treatment and improving outcomes.[4]

Despite remaining the reference standard for diagnosis, liver biopsy cannot be used as a fibrosis screening tool because of its inherent limitations (invasiveness, risk of complications and/or sampling errors, and high costs).[5] Therefore, numerous compound surrogates–based on routine clinical and laboratory parameters–have been developed to screen for fibrosis in patients with chronic liver diseases.[6,7] Among them, the fibrosis-4 index (FIB-4)[8] and NAFLD fibrosis score (NFS)[9] have been extensively used to predict liver fibrosis in large samples of patients with NAFLD.[6,7] However, albeit being inexpensive and readily available even in resource-limited setting, the exact clinical utility of FIB-4 and NFS has not been completely established. Owing to their relatively low positive predictive value (PPV),[6,7] the European Association for the Study of the Liver (EASL) guidelines recommend the use of compound surrogates for excluding, rather confirming, advanced fibrosis.[10] In contrast, the American Association for the Study of the Liver Diseases (AASLD) guidelines maintain the both FIB-4 and NFS are suitable for identifying advanced fibrosis in NAFLD.[11] Another point that remains

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Received on May 19, 2019. Revised on July 16, 2019. Accepted on July 16, 2019. Published online September 19, 2019.
pISSN 1976-2283 eISSN 2005-1212 https://doi.org/10.5009/gnl19173

controversial is the optimal cutoff value that maximizes the diagnostic accuracy of these tools.[12] Finally, the relative performances of each compound surrogate remain unclear, potentially being sample-dependent.[13]

In order to address these issues, we designed the current study to investigate the diagnostic performances of FIB-4 and NFS in detecting (or excluding) biopsy-proven F≥3 in a large sample of Turkish patients with NAFLD.

MATERIALS AND METHODS

1. Patients

This study was designed as a retrospective review of prospectively collected data. The study variables were collected over a 9-year period (from January 2009 to December 2018). A total of 463 consecutive patients aged >18 years with biopsy-proven NAFLD were recruited from the outpatient facilities of the Marmara University School of Medicine. Exclusion criteria were as follows: presence of viral hepatitis, drug-induced liver disease, autoimmune hepatitis, genetic liver diseases, and low platelet count (<100,000/mL). Liver ultrasound was performed in all participants. Liver biopsy was performed in presence of the following indications: (1) evidence of hepatic steatosis on liver ultrasound; (2) abnormal liver enzymes or hepatomegaly or splenomegaly confirmed on imaging studies; and (3) exclusion of secondary causes of hepatic fat accumulation (e.g., significant alcohol consumption [>21 units of alcohol per week for men and >14 units of alcohol per week for women] and previous history of steatogenic drugs use). Liver biopsies were processed by an experienced pathologist as previously described[14] and a histological fibrosis score F≥3 was used to define advanced fibrosis.[15] The pathologist was blinded to FIB-4 and NFS results. The procedures used for data collection have been previously reported in detail.[14,15] The study followed the tenets of the Helsinki Declaration and was approved the local Ethics Committee. Owing to the retrospective nature of the study, the need for informed consent was waived.

2. Calculation of FIB-4 and NFS scores

FIB-4 scores were calculated as previously described[8] using four parameters (platelet count, age, aspartate aminotransferase [AST], and alanine aminotransferase [ALT]). Patients who scored <1.3 and >2.67 on FIB-4 were deemed at low and high risk for advanced fibrosis, respectively.[8] NFS scores were determined using the published formula[9] based on six parameters (age, body mass index, presence of impaired glucose tolerance or diabetes, platelet count, albumin, and AST/ALT ratio). Patients who scored <−1.455 and >0.676 on NFS were deemed at low and high risk for advanced fibrosis, respectively.[9]

3. Statistical analysis

The Kolmogorov-Smirnov test was used to check the normal distribution of continuous data–which are expressed as mean±standard deviation or median (range), as appropriate. Categorical data are given as counts and percentages. Receiver operating characteristic curve analysis was performed to investigate the diagnostic performances of FIB-4 and NFS scores. The optimal binary cutoff values for the two scores in our sample were identified by calculating the Youden's index. The sensitivity, specificity, PPV, and negative predictive value (NPV) for each test were also calculated. All analyses were conducted with the SPSS 24.0 statistical package (IBM Corp., Armonk, NY, USA). A two-tailed p-value <0.05 was considered statistically significant.

Table 1. General Characteristics of the 463 Patients with Biopsy-Proven NAFLD

Factor	Value
Age, yr	46±11
Sex, female/male	243 (52.5)/220 (47.5)
Body mass index, kg/m^2	31.7±5.1
Metabolic syndrome	296 (63.9)
Type 2 diabetes mellitus	175 (37.8)
Hypertension	161 (34.8)
Waist circumference, cm	104±11
AST, U/L	42 (15–302)
ALT, U/L	66 (12–483)
Total cholesterol, mg/dL	212±190
Triglycerides, mg/dL	164 (37–1,107)
HDL cholesterol, mg/dL	44 (18–96)
Platelets, ×10^3/μL	242±67
Hemoglobin, mg/dL	14.4±1.6
Uric acid, mg/dL	6.3±1.6
Glucose, mg/dL	101 (66–307)
Glycated hemoglobin, %	5.7 (3.5–11.1)
HOMA-IR	3.7 (0.3–28.8)
FIB-4 score*	1.05 (0.26–8.22)
Low risk	311 (67.2)
Indeterminate risk	129 (27.8)
High risk	23 (5)
NFS†	−1.73±1.57
Low risk	264 (57)
Indeterminate risk	73 (37.4)
High risk	26 (5.6)

Data are presented as mean±SD, number (%), or median (range).
NAFLD, nonalcoholic fatty liver disease; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; FIB-4, fibrosis-4 index; NFS, NAFLD fibrosis score.
*Patients who scored <1.3 and >2.67 on FIB-4 were considered at low- and high-risk for advanced fibrosis, respectively; †Patients who scored <−1.455 and >0.676 on NFS were regarded as being at low- and high-risk for advanced fibrosis, respectively.

RESULTS

The general characteristics of the 463 study participants are shown in Table 1, whereas their histological features are summarized in Table 2. Advanced fibrosis was present in 81 patients (17.5%).

1. Diagnostic utility of FIB-4

Using the previously published FIB-4 cutoff values for low (<1.3) and high (>2.67) risk for F≥3,[8] we classified 311, 129, and 23 patients in our sample as being at low-, indeterminate-, and high-risk for advanced fibrosis. Based on the results of liver biopsy, we identified histological advanced fibrosis in 29 of the 311 patients (9.3%) classified at low-risk on FIB-4. Advanced fibrosis on histology was also identified in 40 of the 129 patients (31.0%) classified at indeterminate-risk on FIB-4. Finally, 12 of the 23 patients (52.2%) deemed to be at high risk for advanced fibrosis on FIB-4 had a confirmed histological diagnosis of F≥3. The diagnostic performances of the cutoff values for low (<1.3) and high (>2.67) risk for F≥3 are shown in Table 3. The results of receiver operating characteristic curve analysis revealed that the optimal cutoff value for FIB-4 in the identification of advanced fibrosis in our sample was 1.275 (Fig. 1).

2. Diagnostic utility of NFS

Using the previously published NFS cutoff values for low (< −1.455) and high (>0.676) risk for F≥3,[9] we classified 264, 173, and 26 patients in our sample as being at low-, indeterminate-, and high-risk for advanced fibrosis. Based on the results of liver biopsy, we identified histological advanced fibrosis in 29 of the 264 patients (11.0%) classified at low-risk on NFS. Advanced fibrosis on histology was also identified in 40 of the 173 patients (23.1%) classified at indeterminate-risk on NFS. Finally, 12 of the 26 patients (46.1%) deemed to be at high risk for advanced fibrosis on NFS had a confirmed histological diagnosis of F ≥3. The diagnostic performances of the cutoff values for low (<−1.455) and high (>0.676) risk for F≥3 are shown in Table 3. The results of receiver operating characteristic curve analysis revealed that the optimal cutoff value for NFS in the identification of advanced fibrosis in our sample was −1.485 (Fig. 2).

3. Comparison between FIB-4 and NFS

We finally examined the percentage of patients that could avoid liver biopsy in light of a low risk of advanced fibrosis according to the two noninvasive tests under scrutiny. If liver biopsies were performed only in patients with a FIB-4 score above the low cutoff point (≥1.3), 67.1% of biopsies could be avoided. Conversely, if liver biopsies were only performed in patients with an NFS score above the low cutoff point (≥−1.455), 57.0% of biopsies could be avoided. These results indicate that the proportions of patients being at low risk of advanced fibrosis were 67.1% and 57.0% according to FIB-4 and NFS, respectively. The

Table 2. Histopathological Characteristics of the 463 Patients with Biopsy-Proven NAFLD

Characteristic	Value
SAF algorithm classification	
NASH	417 (90.1)
NAFL	46 (9.9)
Grade of steatosis (S) according to SAF score	
S0	0
S1	114 (24.6)
S2	185 (40.0)
S3	164 (35.4)
Grade of activity (A) according to SAF score	
A0	10 (2.2)
A1	32 (6.9)
A2	102 (22.0)
A3	158 (34.1)
A4	161 (34.8)
Stage of fibrosis (F) according to SAF score	
F0	158 (34.1)
F1	144 (31.1)
F2	80 (17.3)
F3	63 (13.6)
F4	18 (3.9)
Grade of ballooning	
0	25 (5.4)
1	215 (46.4)
2	223 (48.2)
Grade of lobular inflammation	
0	31 (6.7)
1	171 (36.9)
2	198 (42.8)
3	63 (13.6)
NAS score (NASH CRN)	5 (1–8)
<3	21 (4.5)
3–4	133 (28.7)
>4	309 (66.8)
Severity of fibrosis	
Significant fibrosis (≥F2)	161 (34.8)
Advanced fibrosis (≥F3)	81 (17.5)
Cirrhosis (F=4)	18 (3.9)
Advanced fibrosis in NASH	79 (18.9)
Advanced fibrosis in NAFL	2 (4.3)

Data are presented as number (%) or median (range).
NAFLD, nonalcoholic fatty liver disease; SAF, steatosis, activity, fibrosis; NASH, nonalcoholic steatohepatitis; NAFL, nonalcoholic fatty liver; NAS, NAFL disease activity score; NASH CRN, NASH clinical research network.

Table 3. Diagnostic Performance of FIB-4 and NFS Indicating Low and High Risk for Advanced Fibrosis in Our Sample (n=463)

Cutoff	Sensitivity (%)	Specificity (%)	FN	FP	PPV	NPV	PLR	NLR
FIB-4								
<1.3	64	74	0.358	0.262	0.342	0.907	2.452	0.485
>2.67	15	97	0.852	0.029	0.522	0.843	5.145	0.877
NFS								
<–1.455	71	63	0.284	0.369	0.291	0.913	1.940	0.450
>0.676	15	96	0.852	0.037	0.462	0.842	4.042	0.884

FIB-4, fibrosis-4 index; NAFLD, nonalcoholic fatty liver disease; NFS, NAFLD fibrosis score; FN, false negative; FP, false positive; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio.

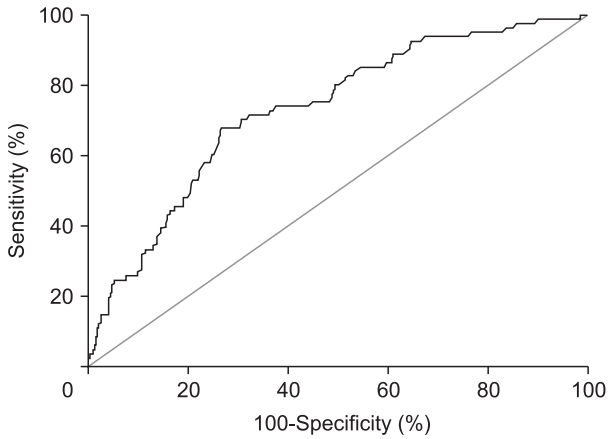

Fig. 1. Receiver operating characteristic curve analysis of the fibrosis-4 index in identifying advanced fibrosis in our sample of patients with biopsy-proven nonalcoholic fatty liver disease (n=463). The results revealed a sensitivity of 68%, a specificity of 73%, and an area under curve of 0.731 (95% confidence interval, 0.672 to 0.790). Diagonal segments are produced by tie.

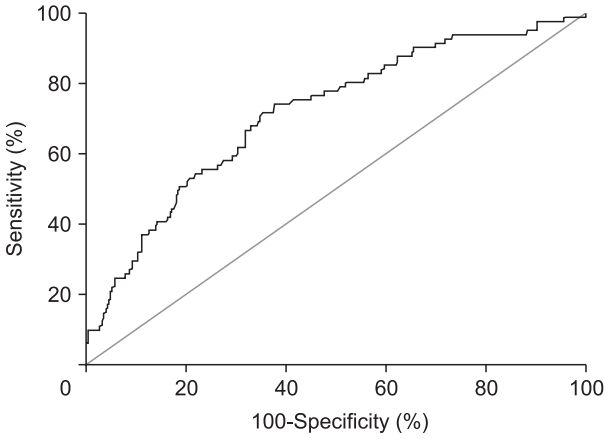

Fig. 2. Receiver operating characteristic curve analysis of the NFS in identifying advanced fibrosis in our sample of patients with biopsy-proven NAFLD (n=463). The results revealed a sensitivity of 74%, a specificity of 62%, and an area under curve of 0.715 (95% confidence interval, 0.652 to 0.777). Diagonal segments are produced by tie.
NAFLD, nonalcoholic fatty liver disease; NFS, NAFLD fibrosis score.

number of patients classified as being at indeterminate risk according to FIB-4 (n=129) was significantly lower than the number obtained when NFS was applied (n=173, p=0.002). However, the proportion of patients with biopsy-proven advanced fibrosis in the indeterminate risk groups was similar for both tests (31.0% for FIB-4 and 23.1% for NFS; p=0.403).

DISCUSSION

There are three principal findings in our study. First, we demonstrated that the clinical utility of both FIB-4 and NFS mainly lies in their ability to exclude, rather than identify, the presence of advanced fibrosis in NAFLD. Second, we identified the optimal cutoff values in our Turkish sample to classify the patients dichotomously (i.e., positive or negative for risk of advanced fibrosis) and calculated the sensitivity, specificity, as well as PPV and NPV of each test. Finally, we have shown that the application of FIB-4 as a screening tool could potentially avoid a larger number of liver biopsies compared with NFS (67.1% vs 57.0%, respectively).

Our results on the clinical usefulness of FIB-4 and NFS being mainly associated with the exclusion, rather than the identification, of advanced fibrosis are in accordance with the EASL[10] but not with the AASLD[11] guidelines. In keeping with previous observations,[16] our data indicate that both scores should not be regarded as diagnostic tests *per se* but rather as screening tools to exclude the diagnosis of advanced fibrosis. This is especially important in resource-limited areas where an expensive procedure like liver biopsy should be limited to selected at-risk cases.

In the original studies of FIB-4 and NFS, the risk of advanced fibrosis was graded into three categories using two different cutoffs.[8,9] This approach leads to the identification of two risk extremes (low- and high-risk) as well as of an intermediate category (in between the two cutoff points) in which the risk is indeterminate. Rather than the traditional ordinal outcomes, we calculated here a single cutoff that produced a dichotomous outcome for each screening tool. The main advantage of reporting an outcome dichotomously (i.e., positive or negative) lies in the possibility to analyze its performance characteristics in terms of sensitivity, specificity, as well as PPV and NPV.[17] Our

data confirm that both FIB-4 and NFS tend to be high-specificity, low-sensitivity tools. The noninvasive identification of liver fibrosis remains a major challenge in the hepatology practice, and numerous unnecessary biopsies are still being performed in patients with NAFLD.[18] An important finding of our study is that FIB-4 could avoid 67.1% of all biopsies as compared with 57.0% of NFS. These observations, coupled with the easier calculation of FIB-4 (four variables) compared with NFS (six variables), clearly support the routine use of the former score over the latter.

Our findings should be interpreted in the context of some limitations. First, we specifically focused on the diagnostic performances of FIB-4 and NFS without considering other compound surrogates (e.g., AST-to-platelet ratio index, BARD index, and Forns index).[19] FIB-4 and NFS were purposely selected for this study because these two tests are recommended by the EASL[10] and AASLD[11] guidelines as noninvasive screening tools for the estimation of advanced liver fibrosis. Second, transient elastrography, a widely used noninvasive imaging tool for detecting hepatic fibrosis,[20,21] was not systematically performed for the purpose of the present investigation. Third, our study was conducted only in Turkish patients and requires replication in independent population. In this regard, it should be noted that the diagnostic performances of compound surrogates may be influenced by potential confounders (e.g., patient age, prevalence of different fibrosis stages, and different NAFLD disease spectrum).[12-19]

These limitations notwithstanding, our results indicate that the main clinical utility of FIB-4 and NFS in patients with NAFLD lies in their ability to exclude, rather than identify, advanced fibrosis. Specifically, the routine application of FIB-4, a simple compound surrogate based on four parameters, is expected to reduce the number of liver biopsy by nearly 70%.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

Study concept and design: E.K., Y.Y. Data acquisition: E.K., H.T.K., C.O.D., C.K. Data analysis and interpretation: E.K., A.B., Y.Y. Drafting of the manuscript; critical revision of the manuscript for important intellectual content: E.K., A.B., H.T.K., C.O.D., C.K., Y.Y. Statistical analysis: A.B. Administrative, technical, or material support; study supervision: Y.Y.

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