

The utility of noninvasive scores in non-alcoholic fatty liver disease patients with normal and elevated serum transaminases

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Abstract

Background and Aim: Noninvasive scores are developed for the estimation of advanced fibrosis, including parameters in addition to transaminases in non-alcoholic fatty liver disease (NAFLD). In this study, we aimed to investigate the diagnostic performances of Fibrosis-4 (FIB-4) and NAFLD Fibrosis Score (NFS) in the estimation of advanced fibrosis comparing patients with normal and elevated transaminases.

Material and Methods: We retrospectively analyzed the prospectively collected data of a total of 407 consecutive patients with biopsy-proven NAFLD. FIB-4 scores of <1.3 and >2.67 or <1.45 and >3.25 indicated a low and high risk for advanced fibrosis, respectively. NFS scores of <1.455 and >0.676 were used to assess low and high risk for advanced fibrosis, respectively.

Results: FIB-4 cutoffs of <1.3 and <1.45 for low risk of advanced fibrosis had a sensitivity of 70% and 54% in patients with elevated transaminases and 70% and 52% in patients with normal transaminases, respectively. The specificities for the cutoffs of >2.67 and >3.25 were 97% and 98% in patients with elevated transaminases and 99% and 100% in patients with normal transaminases, respectively. Concerning NFS, we found similar results.

Conclusion: FIB-4 and NFS showed acceptable diagnostic performance in the exclusion of advanced fibrosis in both populations with normal and elevated transaminases.

Keywords: Biopsy; liver fibrosis; non-alcoholic fatty liver disease; sensitivity and specificity.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is currently the most prevalent chronic liver disease worldwide. NAFLD is estimated to affect 25% of the world population.^[1] Non-alcoholic steatohepatitis (NASH), the subtype of NAFLD, is already among the top etiologies for hepatocellular carcinoma. Due to the close association of NAFLD with obesity and type 2 diabetes mellitus, an increase in incidence and prevalence of

NAFLD is predicted in the recent models. In this respect, NAFLD represents a significant economic and public health burden for the world.^[2]

NAFLD is characterized by evidence of hepatic steatosis and lack of secondary causes of hepatic fat accumulation, such as significant alcohol consumption, long-term use of a steatogenic medication, or hereditary disorder. NAFLD exists on a spectrum from simple steatosis to NASH.^[3-5] The severity of hepatic fibrosis plays especially a significant role in the determination of NAFLD prognosis. NAFLD patients with advanced fibrosis have an increased tendency to progress to cirrhosis, hepatocellular carcinoma, end-stage liver failure and even liver-related mortality, which underlines the importance of timely detection of advanced fibrosis.^[6,7]

To date, liver biopsy remains the reference standard for detecting inflammation and staging fibrosis, which are more likely to be associated with liver-related morbidity and mortality. However, liver biopsy has various main limitations, including high cost, its invasive nature, risk of complications and small size of harvested liver tissue, which may not represent the disease status.^[8,9] Therefore, there is a significant effort to replace liver biopsy with non-invasive modalities, such as clinical and blood-based biochemical testing approaches.^[9,10] Among those, in the recent NAFLD guidelines, Fibrosis-4 Index (FIB-4) and NAFLD Fibrosis Score (NFS) were recommended to use in the estimation of advanced fibrosis.^[3,9]

Clinically, the most common pattern of NAFLD is mildly elevated liver transaminases, although elevated serum transaminase levels poorly correlate with liver histology.^[11] However, serum transaminases are also included in FIB-4^[12] and NFS^[13] as a part of the calculation although there is a lack of evidence whether their diagnostic accuracy differs according to serum transaminase levels. In this study, we aimed to investigate the diagnostic performances FIB-4 and NFS in the identification of advanced fibrosis comparing biopsy-proven NAFLD patients with normal and elevated serum transaminases. We also sought to find optimal cutoffs for our study patients with and without elevated liver enzymes, respectively.

Materials and Methods

Patients

This is a retrospective analysis of the prospectively collected data. A total of 407 consecutive biopsy-proven adults (>18 years) as NAFLD patients who were followed up in outpatient facilities of the Marmara University School of Medicine were recruited for this study. According to our laboratory, an alanine transaminase (ALT) level of ≤ 40 U/L and an

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Table 1. General characteristics of the study patients (n=407)

| | Patients with normal liver enzymes ALT≤40 U/L and AST≤37 U/L (n=107) | Patients with elevated liver enzymes ALT>40 U/L and/or AST>37 U/L (n=300) | Total patients (n=407) | p |
|--|--|---|------------------------|------------------------------|
| Age, median [min.–max.], year | 52 [29–71] | 50 [21–71] | 50 [21–71] | 0.095 ^a |
| Gender, male/female (n) | 38/69 | 130/170 | 168/239 | 0.158 ^b |
| BMI, median [min.–max.], kg/m ² | 32.1 [23.3–56.0] | 31.6 [21.3–52.0] | 31.9 [21.4–56.0] | 0.112 ^a |
| Lean/overweight/obese (n) | 5/28/74 | 12/84/204 | 17/112/278 | 0.907 ^b |
| Metabolic syndrome (yes/no) | 79/28 | 211/89 | 290/117 | 0.492 ^b |
| Type 2 diabetes mellitus (yes/no) | 54/53 | 137/163 | 191/216 | 0.393 ^b |
| Hypertension (yes/no) | 49/58 | 121/179 | 170/237 | 0.325 ^b |
| Hyperlipidaemia (yes/no) | 75/32 | 182/118 | 257/150 | 0.083 ^b |
| Waist circumference, median [min.–max.], cm | 105 [81–147] | 104 [79–146] | 105 [79–147] | 0.117 ^a |
| Hip circumference, median [min.–max.], cm | 112 [92–155] | 109 [84–144] | 110 [84–155] | 0.024^a |
| Albumin, median [min.–max.], mg/dL | 4.6 [3.5–5.3] | 4.6 [3.4–5.6] | 4.6 [3.4–5.6] | 0.330 ^a |
| AST, median [min.–max.], U/l | 25 [11–37] | 47 [22–302] | 40 [11–302] | <0.001^a |
| ALT, median [min.–max.], U/l | 29 [12–39] | 70 [18–343] | 59 [12–343] | <0.001^a |
| ALP, median [min.–max.], U/l | 82 [34–485] | 94 [25–625] | 92 [25–625] | 0.036^a |
| GGT, median [min.–max.], U/l | 35 [9–342] | 54 [10–559] | 49 [9–559] | <0.001^a |
| LDH, median [min.–max.], U/L | 206 [132–732] | 229 [19–969] | 221 [19–969] | 0.003^a |
| Total bilirubin, median [min.–max.], mg/dL | 0.63 [0.21–1.82] | 0.66 [0.12–6.10] | 0.65 [0.12–6.10] | 0.096 ^a |
| Direct bilirubin, median [min.–max.], mg/dL | 0.13 [0.01–0.54] | 0.18 [0.01–1.20] | 0.16 [0.01–1.20] | <0.001^a |
| Total protein, median [min.–max.], g/dL | 7.7 [6.5–9.0] | 7.8 [6.5–9.1] | 7.8 [6.5–9.1] | 0.273 ^a |
| Total cholesterol, median [min.–max.], mg/dL | 211 [79–417] | 210 [74–419] | 210 [74–419] | 0.457 ^a |
| Triglycerides, median [min.–max.], mg/dL | 170 [38–1107] | 162 [37–584] | 163 [37–1107] | 0.498 ^a |
| HDL cholesterol, median [min.–max.], mg/dL | 44 [26–91] | 45 [25–96] | 45 [25–96] | 0.872 ^a |
| LDL cholesterol, median [min.–max.], mg/dL | 127 [35–265] | 134 [28–400] | 132 [28–400] | 0.202 ^a |
| Leucocytes, median [min.–max.], per mL | 7000 [2400–14700] | 6960 [3200–14900] | 6970 [2400–14900] | 0.184 ^a |
| Platelets, median [min.–max.], ×10 ³ per microliter | 225 [77–475] | 230 [89–435] | 229 [77–475] | 0.665 ^a |
| Haemoglobin, median [min.–max.], mg/dL | 13.8 [9.1–17.5] | 14.3 [8.5–18.9] | 14.1 [8.5–18.9] | 0.004^a |
| Glucose, median [min.–max.], mg/dL | 105 [70–240] | 104 [71–307] | 105 [70–307] | 0.850 ^a |
| Creatinine, median [min.–max.], mg/dL | 0.71 [0.45–1.12] | 0.76 [0.41–2.13] | 0.75 [0.41–2.13] | 0.108 ^a |
| BUN, median [min.–max.], mg/dL | 14 [6–29] | 15 [7–36] | 14 [6–36] | 0.367 ^a |
| Uric acid, median [min.–max.], mg/dL | 6 [2.1–8.2] | 6.4 [0.7–11.1] | 6.2 [0.7–11.1] | 0.184 ^a |
| HbA1c, median [min.–max.], % | 5.9 [4.5–8.9] | 5.9 [4.0–11.1] | 5.9 [4.0–11.1] | 0.555 ^a |
| HOMA-IR, median [min.–max.] | 4.65 [1.06–15.53] | 5.29 [1.0–28.76] | 4.90 [1.0–28.76] | 0.084 ^a |

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; ALP: Alkaline phosphatase; GGT: Gama glutamyl transferase; LDH: Lactate dehydrogenase; HDL: High density lipoprotein; LDL: Low density lipoprotein; SD: Standard deviation; BUN: Blood urea nitrogen; HbA1c: HemoglobinA1c; HOMA-IR: Homeostatic model assessment for insulin resistance; Min.: Minimum; Max. Maximum. Statistical tests, a: Mann-Whitney U Test, b: Chi-Square Test.

aspartate transaminase (AST) level of ≤37 U/L were defined as normal. An ALT level of >40 U/L and/or AST level of >37 U/L were considered elevated serum transaminase levels. Exclusion criteria were explained in detail previously.^[14] The groups were well-matched concerning age, gender and body mass index (BMI). Liver biopsies were evaluated by a single pathologist expertized in the liver as previously described^[15] and a histological fibrosis score F≥3 was used to define advanced fibrosis.^[16] The pathologist was blinded to FIB-4 and NFS results.

Calculation of the FIB-4 and NFS

FIB-4 scores were calculated using four parameters (platelet count,

age, AST, ALT)^[17] and NFS six parameters (age, BMI, presence of impaired glucose tolerance or diabetes, platelet count, albumin and AST/ALT ratio)^[13] following the published formulas. For FIB-4, patients who scored <1.3^[12] and <1.45^[17] were defined as low risk for advanced fibrosis and >2.67^[12] and >3.25^[17] as high risk for advanced fibrosis. According to NFS, patients who scored <-1.455 and >0.676 were defined as low and high risk for advanced fibrosis, respectively.^[13]

Statistical Analysis

The normal distribution of continuous data was assessed using the Shapiro-Wilk test. The normally distributed continuous data were ex-

Table 2. Histological characteristics of the study patients

| | Patients with normal liver enzymes ALT≤40 U/L and AST≤37 U/L (n=107) | Patients with elevated liver enzymes ALT>40 U/L and/or AST>37 U/L (n=300) | Total patients (n=407) | p |
|---|--|---|---------------------------|---------------------|
| NAS score (NASH CRN), median [min.–max.] | 4 [1–8] | 6 [1–8] | 5 [1–8] | <0.001 ^a |
| SAF algorithm classification, NASH/NAFL, (n) | 92/15 | 280/20 | 372/35 | 0.033 ^b |
| Grade of steatosis (S) according to the SAF score: S0/S1/S2/S3 (n) | 0/47/41/19 | 0/58/112/130 | 0/105/153/149 | <0.001 ^b |
| Stage of activity (A) according to the SAF score: A0/A1/A2/A3/A4, (n) | 3/11/36/32/25 | 3/14/55/100/128 | 6/25/91/132/153 | <0.001 ^b |
| Stage of fibrosis (F) according to the SAF score: F0/F1/F2/F3/F4, (n) | 48/22/14/16/7 | 58/111/64/51/16 | 106/133/78/67/23 | <0.001 ^b |
| Mild disease/severe disease (n) | 13/94 | 16/284 | 29/378 | 0.033 ^b |
| Significant fibrosis (F≥2), (n) | 37 | 131 | 168 | 0.101 ^b |
| Advanced fibrosis (F≥3), (n) | 23 | 67 | 90 | 0.858 ^b |
| Cirrhosis (F=4), (n) | 7 | 16 | 23 | 0.642 ^b |

NASH: Non-alcoholic steatohepatitis; NAFL: Non-alcoholic fatty liver; NAS: Non-alcoholic fatty liver disease activity score; CRN: Clinical Research Network; Min.: Minimum; Max. Maximum. Statistical tests, a: Mann-Whitney U Test, b: Chi-Square Test.

pressed as mean±standard deviation and compared with the Independent Sample t-test. Nonnormally distributed continuous data were expressed as median [minimum-maximum] and compared using the Mann-Whitney U test. Categorical data were given as count and percentages and analyzed using the chi-square test. Receiver operating characteristics (ROC) analysis was carried out to calculate the area under the curve to identify the optimal cutoff value for FIB-4 and NFS. The highest value for Youden's index was defined as the optimal cutoff. The sensitivity, specificity, positive predictive value, and negative predictive value for each test were also presented. Cohen's kappa was used as a measure of agreement between the noninvasive test and the reference standard. All analyses were performed using the SPSS 24.0 statistical package (IBM, Armonk, NY, USA). A two-tailed p value <0.05 was considered statistically significant.

Ethics

This study was conducted in adherence to the Helsinki Declaration and was approved by the Marmara University ethics committee. Due to the retrospective nature of this study, the need for informed consent was waived. This work has been supported Marmara University Scientific Research Projects Coordination Unit under grant number: SAG-C-TUP-150218-0033.

Results

From 407 biopsy-proven NAFLD patients, 107 patients (26.3%) had normal and 300 patients (73.7%) elevated serum transaminases. General characteristics of the study patients were presented in Table 1, comparing patients with normal and elevated serum transaminase levels. In Table 2, the histological characteristics of the patients were summarized. Among patients with normal serum transaminases, 23 patients (21.5%) had advanced fibrosis, whereas among patients with elevated serum transaminases were 67 (22.3%). The prevalence of advanced fibrosis among those groups did not significantly differ (p=0.858).

In patients with normal serum transaminase levels, using the previously published cutoff values for FIB-4 (<1.3 and <1.45 for low risk of advanced fibrosis and >2.67 and >3.25 for high risk of advanced

fibrosis), we classified 73 (68.2%) and 79 (73.8%) patients as low risk, 30 (28.0%) and 27 (25.2%) patients as indeterminate risk and 4 (3.7%) and 1 (0.9%) patients as high risk of advanced fibrosis, respectively. Considering NFS, 41 patients (38.3%) were classified as low risk, 52 (48.6%) as indeterminate and 14 (13.1%) as high risk for advanced fibrosis. Using the cutoff values for FIB-4 (<1.3 and <1.45 for low risk of advanced fibrosis and >2.67 and >3.25 for high risk of advanced fibrosis) in patients with elevated serum transaminases, we identified 171 (57%) and 210 patients (70%) as low risk, 111 (37%) and 75 patients (25%) as indeterminate risk and 18 (6%) and 15 patients (5%) as high risk for advanced fibrosis, respectively. For NFS, we classified 159 patients (53%) as low, 127 (42.3%) as indeterminate and 14 (4.7%) as high risk of advanced fibrosis.

The diagnostic performances of FIB-4 and NFS in patients with normal and elevated serum transaminases were presented in Table 3 and Table 4, respectively. Accordingly, especially cutoffs indicating a high risk of advanced fibrosis had a relatively good diagnostic performance in exclusion of advanced fibrosis considering the high specificity values for both of the groups, including all the cutoffs of FIB-4 and NFS. However, low sensitivity values indicated low diagnostic performance in the identification of advanced fibrosis. Concerning optimal cutoff values, we detected the best cutoff values at >1.2 and >1.3 for FIB-4 for elevated and normal transaminase levels, whereas for NFS at >-1.483 and >-0.587, respectively. We found overall suboptimal agreement with the noninvasive tests and reference standard considering low kappa values both for the cutoffs recommended in the literature and optimal cutoffs revealed in our study.

Discussion

In this study, to our knowledge, we compared for the first time the diagnostic performances of FIB-4 and NFS using different cutoff values recommended in the literature between patients with normal and elevated serum transaminase levels, and we found similar diagnostic performance in both of the groups. The optimal FIB-4 cutoff value revealed in our population for patients with normal serum transaminases was >1.3 for the identification of advanced fibrosis, which was coherent

Table 3. Diagnostic performance of the FIB-4 in patients with normal (n=107) and elevated (n=300) serum transaminases in assessment of advanced fibrosis

| | Sensitivity (%) | Specificity (%) | AUC | 95% CI | FN | FP | PPV | NPV | PLR | NLR | Kappa |
|--|-----------------|-----------------|-------|-------------|-------|-------|-------|-------|--------|-------|-------|
| Patients with normal serum transaminases (n=107) | | | | | | | | | | | |
| FIB-4 cutoffs | | | | | | | | | | | |
| >1.3 | 70 | 79 | – | – | 0.304 | 0.214 | 0.471 | 0.904 | 3.246 | 0.387 | 0.410 |
| >2.67 | 13 | 99 | – | – | 0.870 | 0.012 | 0.750 | 0.806 | 10.957 | 0.880 | 0.169 |
| >1.45 | 52 | 81 | – | – | 0.478 | 0.190 | 0.429 | 0.861 | 2.739 | 0.591 | 0.307 |
| >3.25 | 0 | 100 | – | – | 0.957 | 0 | 1 | 0.792 | – | 0.792 | 0.067 |
| Optimal FIB-4 cutoff | | | | | | | | | | | |
| >1.3 | 70 | 79 | 0.757 | 0.644–0.870 | 0.304 | 0.214 | 0.471 | 0.904 | 3.246 | 0.387 | 0.410 |
| Patients with elevated serum transaminases (n=300) | | | | | | | | | | | |
| FIB-4 cutoffs | | | | | | | | | | | |
| >1.3 | 70 | 65 | – | – | 0.299 | 0.352 | 0.364 | 0.883 | 1.993 | 0.461 | 0.263 |
| >2.67 | 18 | 97 | – | – | 0.821 | 0.026 | 0.667 | 0.805 | 6.955 | 0.843 | 0.207 |
| >1.45 | 54 | 77 | – | – | 0.463 | 0.232 | 0.400 | 0.852 | 2.318 | 0.602 | 0.272 |
| >3.25 | 15 | 98 | – | – | 0.851 | 0.021 | 0.667 | 0.800 | 6.955 | 0.869 | 0.177 |
| Optimal FIB-4 cutoff | | | | | | | | | | | |
| >1.2 | 81 | 58 | 0.744 | 0.682–0.806 | 0.194 | 0.416 | 0.358 | 0.913 | 1.936 | 0.332 | 0.269 |

AUC: Area under curve; CI: Confidence interval; FN: False negative; FP: False positive; PPV: Positive predictive value; NPV: Negative predictive values; PLR: Positive likelihood ratio; NLR: Negative likelihood ratio.

Table 4. Diagnostic performance of the NFS in patients with normal (n=107) and elevated (n=300) serum transaminases in assessment of advanced fibrosis

| | Sensitivity (%) | Specificity (%) | AUC | 95% CI | FN | FP | PPV | NPV | PLR | NLR | Kappa |
|--|-----------------|-----------------|-------|-------------|-------|-------|-------|-------|--------|-------|-------|
| Patients with normal serum transaminases (n=107) | | | | | | | | | | | |
| NFS cutoffs | | | | | | | | | | | |
| >1.455 | 87 | 45 | – | – | 0.130 | 0.548 | 0.303 | 0.927 | 1.588 | 0.288 | 0.192 |
| >0.676 | 26 | 91 | – | – | 0.739 | 0.095 | 0.429 | 0.817 | 2.739 | 0.817 | 0.193 |
| Optimal NFS cutoff | | | | | | | | | | | |
| >0.587 | 78 | 73 | 0.751 | 0.641–0.860 | 0.217 | 0.274 | 0.439 | 0.924 | 2.858 | 0.299 | 0.396 |
| Patients with elevated serum transaminases (n=300) | | | | | | | | | | | |
| NFS cutoffs | | | | | | | | | | | |
| >1.455 | 76 | 61 | – | – | 0.239 | 0.395 | 0.357 | 0.898 | 1.928 | 0.395 | 0.261 |
| >0.676 | 16 | 99 | – | – | 0.836 | 0.013 | 0.786 | 0.804 | 12.751 | 0.847 | 0.211 |
| Optimal NFS cutoff | | | | | | | | | | | |
| >1.483 | 79 | 59 | 0.734 | 0.668–0.799 | 0.209 | 0.408 | 0.358 | 0.908 | 1.940 | 0.353 | 0.268 |

AUC: Area under curve; CI: Confidence interval; FN: False negative; FP: False positive; PPV: Positive predictive value; NPV: Negative predictive values; PLR: Positive likelihood ratio; NLR: Negative likelihood ratio.

with the cutoff reported in the literature.^[12] The best agreement was also detected at this cutoff for patients with normal serum transaminases (kappa=0.410), whereas a FIB-4 cutoff of >1.2 showed the best agreement in patients with elevated serum transaminases (kappa=0.269). Additionally, for both FIB-4 and NFS, we concluded that noninvasive tests were useful in exclusion of advanced fibrosis rather than identification of it as recommended in the guideline of European Association for the Study of the Liver,^[3] but in contrast with the guideline of American Association for the Study of Liver Diseases.^[9]

The prevalence of NASH with advanced fibrosis is not well defined, and current estimates tend to underestimate the true prevalence given

the assessment of advanced fibrosis was estimated with noninvasive methods rather than using reference standard.^[18–20] However, from Turkey, advanced fibrosis was reported to affect a substantial number of patients (17.3%) with biopsy-proven NAFLD, which also shows the poor diagnostic accuracy of noninvasive tests in the identification of advanced fibrosis.^[14] Therefore, liver biopsy remains still the reference standard for the identification of histological features,^[21,22] especially the detection of advanced fibrosis represents a clinical significance, which is associated with a higher risk of liver-related morbidity and mortality. Therefore, it becomes a major focus for drug trials among patients with NASH.^[23–27] In other respects, it is of critical importance

to exclude those patients with advanced fibrosis accurately to avoid unnecessary liver biopsy.^[28]

Although both FIB-4 and NFS showed similar diagnostic performance concerning the exclusion of advanced fibrosis, FIB-4 can be used over NFS because of its slightly better performance and easier application using only four parameters in clinical practice, which was also reported previously.^[29] Additionally, following first-line triaging of NAFLD patients with advanced fibrosis with FIB-4 and NFS, performing Fibroscan to those patients were found to increase diagnostic accuracy,^[30] which was considered as a useful diagnostic tool in screening liver involvement in patients with NAFLD.^[31] In this scenario, patients who classified as low risk for advanced fibrosis must be managed in primary care, whereas patients with high risk should be directly referred to secondary care. However, patients who were classified as indeterminate risk according to FIB-4 or NFS should undergo Fibroscan examination.^[32]

Our study has several limitations. Firstly, we performed this study focusing only on FIB-4 and NFS without investigating other noninvasive scores.^[29] Secondly, we defined normal serum transaminase levels according to our laboratory, which may not be compatible with other laboratories' diagnostic values.

Conclusion

In conclusion, FIB-4 and NFS showed acceptable diagnostic performance in the exclusion of advanced fibrosis rather than detection of it. Among those, FIB-4 can be the noninvasive diagnostic test of choice in clinical routine, considering its easier calculation with fewer parameters.

Ethics Committee Approval: This study was conducted in adherence to the Helsinki Declaration and was approved by the Marmara University ethics committee (date: 01.11.2019, number: 09.2019.944).

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Author Contributions: EK, FE, YY: Conception and design of the study; EK, AB: Data acquisition; EK, AB, YY: Statistical analysis; EK, AB, FE, YY: Data analysis and drafting of the manuscript. All authors critically revised the manuscript and approved the final version.

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