ORIGINAL ARTICLE

Blood Biomarkers of Fibrosis as Alternatives to FibroScan® in Metabolic Dysfunction-associated Fatty Liver Disease: A Single-center Comparative Analysis

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Abstract

Background: Liver fibrosis worsens prognosis in metabolic dysfunction-associated fatty liver disease (MAFLD). FibroScan® is the most widely used noninvasive tool for evaluating fibrosis, but performing this assessment requires specialized equipment and expertise. This study aimed to assess the potential of four additional noninvasive techniques for diagnosing liver fibrosis that rely on routine laboratory measurements, that is, fibrosis (FIB)-4 score, FIB-5 score, aspartate aminotransferase (AST)/alanine transaminase (ALT) ratio, and the aspartate aminotransferase to platelet ratio index (APRI).

Methods: This study was performed following a cross-sectional observational design at a tertiary care hospital in India. The study included adult patients who were observed to have elevated serum AST and ALT levels and fatty deposition on ultrasonography, as these indicate a risk for liver fibrosis, that is, MAFLD or metabolic dysfunction-associated steatohepatitis. The specificity and sensitivity of FIB-4, FIB-5, APRI, and AST/ALT ratio were compared with those of FibroScan® (FibroScan® 502, Echosens, Paris, France).

Results: Among the alternative noninvasive methods, FIB-4 had the highest specificity (78%) and sensitivity (85%) that were closest to the specificity (88%) and sensitivity (92%) of FibroScan®. FIB-5 and APRI demonstrated moderate sensitivity (80% and 76%, respectively) and specificity (75 and 70%, respectively). The AST/ALT ratio had relatively poor diagnostic capability, with a specificity of 60% and sensitivity of 65%. The area under the curve (AUC) for the methods being compared was 0.82 (FIB-4), 0.79 (FIB-5), 0.74 (APRI), and 0.65 (AST/ALT ratio).

Conclusion: FibroScan® is the preferred option for evaluating liver fibrosis in patients with MAFLD. However, when unavailable, FIB-4 may be the next most reliable alternative for identifying or excluding advanced fibrosis. Other methods (FIB-5, APRI, and AST/ALT) are less accurate.

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Introduction

Metabolic dysfunction-associated fatty liver disease (MAFLD) is a common and growing public health concern, with almost one in three individuals diagnosed with this condition worldwide.[1] MAFLD and its progressive form, metabolic dysfunction-associated steatohepatitis (MASH), are typically characterized by fibrosis of liver tissue.[2] Stage 4 fibrosis is associated with a 3.4-fold increase in the risk of all-cause mortality and an 11.1-fold increase in the risk of death due to progression of liver disease.[3] Early detection of patients with or at risk for advanced fibrosis is essential for timely intervention and disease progression prevention in primary and endocrine care settings, where MAFLD prevalence is high.[4,5]

Biopsy is the gold standard for diagnosing the degree of fibrosis by assessing the deposition of extracellular matrix components as well as the degradation and remodeling of the matrix.[6] However, this test cannot be used ubiquitously in all patients to assess the extent of fibrosis.[7] The first limitation is that of procedural complications. One in five patients experiences pain, and due to the invasive nature of the procedure, bleeding complications and consequently death are a possibility. Another limitation is that of sampling error because only a fraction (1:50,000) of the liver sample is excised for analysis. A third important limitation is that a high level of expertise is necessary to perform a liver biopsy, paving the way for intra- and interobserver variability, especially in terms of evaluating inflammatory activity. Therefore, performing a liver biopsy may not be feasible in all clinical settings and may be especially challenging in resource-limited settings.

Several arguments against liver biopsy have been made, and alternative noninvasive techniques for screening liver fibrosis have been suggested. Transient elastography (TE) applies a low-frequency (50 Hz) ultrasound elastic shear wave to assess the stiffness of liver tissue as a proxy for liver fibrosis. This test, performed using the FibroScan® device, has a coverage that is more than 100 times that of a biopsy.[7] Magnetic resonance elastography is another noninvasive method for evaluating the degree of liver fibrosis, reportedly offering greater accuracy than FibroScan®; however, high operational costs limit its use in most clinical settings.

With a view to eliminating any equipment cost, some researchers have proposed simple calculation-based metrics, including nonalcoholic fatty liver disease fibrosis score (NFS), fibrosis-4 (FIB-4) score, fibrosis-5 (FIB-5) score, aspartate aminotransferase (AST)-to-platelet ratio index (APRI), enhanced liver fibrosis (ELF) score, and AST/alanine transaminase (ALT) ratio to estimate the risk of liver fibrosis with reasonable accuracy. The FIB-4 index, initially reported in 2006 to evaluate hepatic fibrosis in patients with HIV and HCV coinfection, has subsequently been adapted for broader use in assessing liver fibrosis.[8] Due to the inclusion of age as a factor, the accuracy of the FIB-4 index in estimating liver fibrosis may be compromised in older patients with liver disease.[9] The FIB-5 is a modified version of FIB-4 that enables estimation of liver fibrosis based on five blood parameters instead of four and is reported to be superior to FIB-4 in distinguishing between significant and nonsignificant liver fibrosis.[10] Evidence comparing the sensitivity and specificity of these alternative methods with those of FibroScan® is lacking, especially in the resource-constrained settings in India.

This study assessed diagnostic accuracy, specificity, and sensitivity of FIB-4, FIB-5,

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APRI, and AST/ALT ratio for evaluating the risk of developing liver fibrosis in comparison to FibroScan® among patients with suspected MAFLD at a single center in India. The study population comprised individuals who were observed to have fatty liver on ultrasonography, as these are the patients who are at greater risk for liver fibrosis, that is, MAFLD and MASH. Identification of alternatives to FibroScan® in these individuals will facilitate the screening of MAFLD in its early stages using methods of equivalent efficacy compared to FibroScan®.

Methods

Study Design and Settings

This was a cross-sectional observational study conducted at a tertiary care hospital in Jammu, India. The STROBE guidelines were followed for reporting the findings.

Study Population

Patients above 18 years with elevated serum liver enzymes (AST and ALT) and fatty liver detected *via* abdominal ultrasound examination, which was suspected to indicate MAFLD or MASH, were included. Ultrasonography demonstrated (A) normal liver echogenicity, (B) grade 1 fatty liver characterized by increased liver echogenicity, (C) grade 2 fatty liver where the echogenic liver obscures the echogenic walls of the portal venous branches, or (D) grade 3 fatty liver in which the outline of the diaphragm is obscured.[11] Patients with fatty liver included those with grade 1–3 fatty liver. To facilitate comparison between the fibrosis assessment techniques, patients were enrolled if they had undergone a FibroScan® analysis. Biopsy was not included as a criterion due to the impracticality of conducting biopsies on every individual suspected of having liver fibrosis in the Indian context.

Patients were excluded from the study if they had a history of alcohol use, other chronic liver conditions (including viral hepatitis and autoimmune liver disease), genetic predisposition diseases that can cause liver damage like alpha-1 antitrypsin deficiency, those with certain forms of hepatic dysfunction, such as hemochromatosis, those with malignancies or other serious comorbidities that could affect liver function, and those who reported a history of treatment with drugs with hepatotoxic potential.

All participants provided written informed consent prior to inclusion in the study.

Study Variables and Measurements

Transient Elastography (FibroScan®)

Each participant received FibroScan® analysis (FibroScan® 502, Echosens, Paris, France) to measure liver stiffness, which was utilized as the benchmark for evaluating liver fibrosis. The FibroScan® analysis was conducted by an expert radiologist using an M probe for individuals with a body mass index (BMI) <25 kg/m^2 (nonobese individuals) and an XL mode for those with a BMI ≥25 kg/m^2 (obese individuals).[12,13] The patients were asked to fast overnight or the test was conducted at least a few hours after a meal. Imaging was conducted with the patient lying in a supine position. Using the A-mode images from the FibroScan® device, the operator identified a liver section at least 6 cm thick, free of large vascular structures. The measurement depth during image acquisition ranged from 25 to 45 mm. Liver fibrosis severity was determined according to the reported liver stiffness measurement cutoff values of 1.0–6.0 kPa (F0: no fibrosis), 6.1–7.0 kPa (F1: mild fibrosis), 7.1–9.0 kPa (F2: moderate fibrosis), 9.1–10.3 kPa (F3: severe fibrosis), and ≥ 10.4 kPa (F4: cirrhosis).[14]

All other methods assessed in this study were compared with values obtained with FibroScan® as a reference.

Blood Parameters

The levels of ALT, ALP, AST, and albumin were determined using an autoanalyzer, and platelet counts were estimated using the hydrodynamic focusing method. The FIB-4 index was calculated based on patient age, ALT level, AST level, and platelet count as follows[10]:

Age (years) × AST (IU/L) / [platelet count (10^9/L) × ALT (IU/L)$^{1/2}$]

The FIB-5 index was calculated based on the AST/ALT ratio, albumin, ALP, and platelet count as follows:

Albumin (gm/L) × 0.3 + platelet count (10^9/L) × 0.05 − ALP (IU/L) × 0.014 + AST/ALT ratio × 6 + 14

The APRI was calculated based on AST level and platelet count as follows[15]:

[AST (IU/L) / ULN (IU/L)] × 100 / platelet count (10^9/L)

ULN: upper limit of normal for AST.

Sample Size

A sample size of 300 patients was determined to yield reliable data for thorough statistical analysis and effective comparisons.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows (version 28.0, IBM Corp., Armonk, NY) and R (R Core Team, 2021; R Foundation for Statistical Computing, Vienna, Austria). Receiver operating characteristic (ROC) curves were plotted to determine the area under the curve (AUC) for each fibrosis assessment method as an accuracy measure. Specificity and sensitivity for each method were also calculated.

Results

Patient Characteristics

The study included 300 patients with MAFLD or MASH. Table 1 displays the demographic and clinical characteristics of the study population. Most patients had comorbidities such as T2DM (70%), metabolic syndrome (65%), and obesity (50%).

Comparison of Diagnostic Methods

The specificity and sensitivity of the various noninvasive scoring methods were evaluated. The pooled data are shown in Figure 1.

FibroScan® demonstrated the highest sensitivity, specificity, and accuracy (based on the AUC). Among the alternative methods, FIB-4 showed the best diagnostic performance, followed by FIB-5 and APRI. The AST/ALT ratio exhibited the lowest sensitivity, specificity, and accuracy among the compared methods.

In terms of accuracy, based on the AUC, a similar pattern was noted. FIB-4 had the best AUC of 0.82, which was closest to the AUC for FibroScan®, that is, 0.90 (Fig. 2). The AST/ALT ratio had the least AUC of 0.65.

Discussion

It is well established that fibrosis of liver tissue has a substantial impact on the prognosis of patients with MAFLD. Patients are being increasingly involved in healthcare decisions, and the cost of diagnostic procedures can

Table 1: Demographic and clinical characteristics of the study population

Characteristic	*Overall population (N = 300)*
Mean age, years	48.0
Sex, *n* (%)	
Male	180 (60.0)
Female	120 (40.0)
Body mass index, mean, kg/m^2	32.0
Comorbidities, *n* (%)	
T2DM	210 (70.0)
Metabolic syndrome	195 (65.0)
Obesity	150 (50.0)

T2DM, type 2 diabetes mellitus

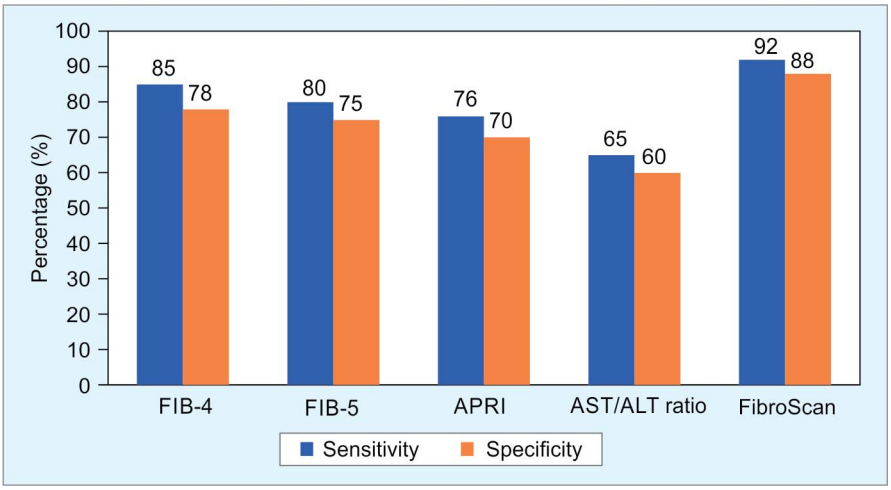

Fig. 1: Comparative sensitivity and specificity of the various noninvasive scoring methods; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; FIB, fibrosis index

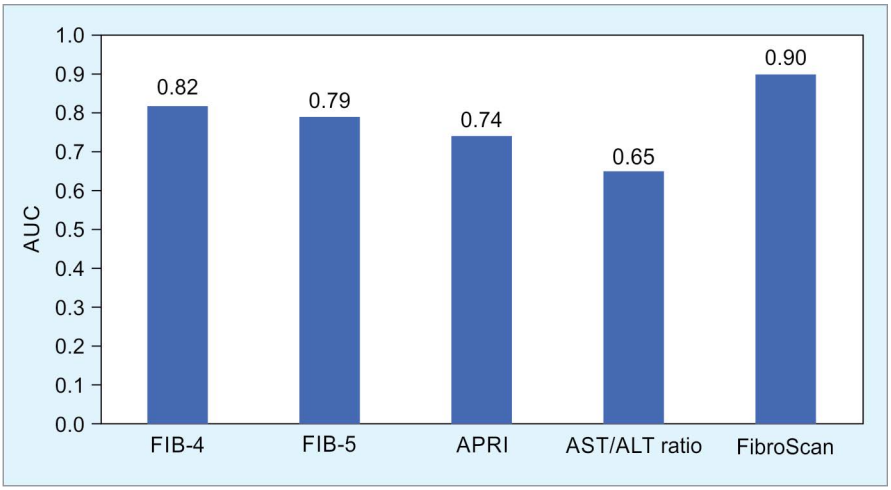

Fig. 2: Comparative area under the receiver operating characteristics curve of the various noninvasive scoring methods; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; AST, aspartate aminotransferase; FIB, fibrosis index

influence their choices. While noninvasive procedures are preferred by both patients and clinicians, selecting the most clinically appropriate diagnostic tool for liver fibrosis from available options remains crucial. FibroScan® is frequently employed to detect the existence and degree of liver fibrosis; however, not all facilities may be equipped with the appropriate systems to perform a FibroScan® assessment. Although this technique is noninvasive, qualified professionals are needed to perform this assessment. Therefore, in this study, we evaluated the possibility of using other methods of diagnosing liver fibrosis and compared it with four other noninvasive tools that rely on routine laboratory measurements, that is, FIB-4, FIB-5, APRI, and AST/ALT ratio. The study findings show that FIB-4 demonstrated the highest specificity and sensitivity that closely matched the specificity and sensitivity of FibroScan®. FIB-5 and APRI demonstrated moderate sensitivity and specificity, and the AST/ALT ratio had relatively poor diagnostic capability.

Chronic hepatic inflammation drives liver fibrosis, which then progresses to liver cirrhosis. Therefore, early diagnosis through screening programs is critical. Some studies have reported that around 18%–27% of patients at risk for liver disease have undiagnosed liver fibrosis or cirrhosis.[16] Since liver biopsy is considered the definitive method for diagnosing liver fibrosis, any noninvasive test must demonstrate comparable diagnostic accuracy to be deemed a viable alternative to this invasive procedure. In a study performed in the United Kingdom, individuals with known risk factors for chronic liver disease underwent TE, an imaging technique used to identify liver fibrosis by assessing liver stiffness. It was noted that TE had a specificity and sensitivity of 97 and 86% in diagnosing liver fibrosis.[17] In the present study, we found that FibroScan®, which is a procedure based on TE, had a specificity and sensitivity of 88 and 92%. It was still the best among all the compared noninvasive methods of assessing liver fibrosis. However, not all hospitals or clinics may be in a position to use this technique due to a lack of technical and human resources. Moreover, not all patients may benefit from this assessment. For example, patients who are morbidly obese or have excessive fat deposition around the chest area and those who have liver ascites are deemed ineligible to undergo FibroScan® analysis. The main reason for excluding these patients is that the test results with FibroScan® are not reliable in these patient groups.[18] In such situations, it is particularly helpful to use indirect methods of assessing liver fibrosis.

The FIB-4 score is one type of indirect approach for evaluating liver fibrosis.[19] It is based on four factors: patient age, platelet count, ALT level, and AST level. The effectiveness of FIB-4 in screening individuals at high risk for liver fibrosis related to MAFLD has been demonstrated.[19,20] Moreover, because this technique is based on a simple calculation using routine laboratory parameters, there is no question of its availability even in resource-limited settings. Studies suggest that patients suspected of liver fibrosis from the FIB-4 score may be selected for testing by TE or the ELF test. The FIB-4 index has been proposed as a predictor of chronic kidney disease, cardiovascular disease, and extrahepatic malignancies, as well as liver-related mortality and hepatocellular carcinoma.[21] In the present study, FIB-4 was next best at diagnosing liver fibrosis after FibroScan®. The simplicity and cost-effectiveness of FIB-4 make it an ideal test during the first-step screening for liver fibrosis.

The FIB-5 score is similar to FIB-4, but it is based on the AST/ALT ratio, platelet count, ALP, and albumin. One study comparing these two scores found that FIB-5 had greater specificity than FIB-4 in differentiating between significant and nonsignificant fibrosis in individuals with chronic hepatitis B.[22] Another study reported that the FIB-5 score was superior to the FIB-4 score in distinguishing significant from nonsignificant fibrosis in patients with chronic hepatitis C.[10] In the present study, both FIB-4 and FIB-5 had similar sensitivity (85 and 80%, respectively) and specificity (78 and 75%, respectively). While this study did not determine the positive and negative predictive values, prior research has indicated that both FIB-4 and FIB-5 are effective in ruling out advanced liver

fibrosis in patients with MAFLD.[23] They can be used as an alternative to FibroScan® when resources to perform a FibroScan® assessment are limited.

Some researchers have combined APRI and FIB-4 to predict cirrhosis in individuals with chronic hepatitis C. In one study, this combination of markers was considered a suitable alternative to liver stiffness measurements using FibroScan®, which may not be widely available in rural areas.[24] However, another study on patients with chronic hepatitis B showed that while these scores correlated with Ishak stage determined through liver biopsy, they could not accurately differentiate among the various stages of fibrosis.[25] The authors of that study suggested that APRI and FIB-4 are not suitable for determining the extent of liver damage in patients with chronic hepatitis B. In a study involving Portuguese patients with MAFLD and without decompensated cirrhosis, APRI and FIB-4, along with other methods, were deemed effective for excluding advanced fibrosis in clinical settings and could be incorporated into referral or follow-up programs for this group.[26]

Overall, based on the literature reports, we see that the ability of these alternative noninvasive tools for assessing liver fibrosis varies according to the underlying liver condition. While they may not always be suitable for patients with hepatitis B or C, these methods are viewed as a viable alternative to FibroScan® in cases of MAFLD. This study has a few limitations. First, we have not determined the positive and negative predictive values for the different tests. Therefore, we could not compare the tests based on their associated rates of false positives, false negatives, and misclassification. Second, we did not determine the fibrosis stage by FibroScan®. It is possible that some of the alternative tools may be more useful before the patient develops advanced fibrosis than after. The lack of symptoms in MAFLD complicates the assessment of fibrosis stages. In the future, we intend to compare the different noninvasive methods examined in this study, stratifying patients according to their liver fibrosis stage as evaluated by FibroScan®.

Conclusion

In resource-constrained environments where FibroScan® is unavailable or cost-prohibitive, it is crucial to determine if alternative methods can reliably assess liver fibrosis, particularly in identifying advanced disease. FIB-4 most accurately mirrored FibroScan® results in identifying and excluding liver fibrosis. FIB-5 and APRI showed moderate accuracy, while the AST/ALT ratio performed poorly. The study reinforces the superior diagnostic capability of FibroScan®, but the use of the FIB-4 method may be considered when FibroScan® is not immediately available or essential.

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