Original Article

# Validation of FIB-6 score in assessment of liver fibrosis in chronic hepatitis B

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## Abstract

**Background:** We recently developed a simple novel index called fibrosis 6 (FIB-6) using machine learning data analysis. We aimed to evaluate its performance in the diagnosis of liver fibrosis and cirrhosis in chronic hepatitis B (CHB).

**Methods:** A retrospective observational analysis of data was obtained from seven countries (Egypt, Kingdom of Saudi Arabia (KSA), Turkey, Greece, Oman, Qatar, and Jordan) of CHB patients. The inclusion criteria were receiving an adequate liver biopsy and a complete biochemical and hematological data. The diagnostic performance analysis of the FIB-6 index was conducted and compared with other non-invasive scores.

**Results:** A total of 603 patients were included for the analysis; the area under the receiver operating characteristic curve (AUROC) of FIB-6 for the discrimination of patients with cirrhosis (F4), compensated advanced chronic liver disease (cACLD) (F3 and F4), and significant fibrosis (F2–F4) was 0.854, 0.812, and 0.745, respectively. The analysis using the optimal cut-offs of FIB-6 showed a sensitivity of 70.9%, specificity of 84.1%, positive predictive value (PPV) of 40.3%, and negative predictive value (NPV) of 95.0% for the diagnosis of cirrhosis. For the diagnosis of cACLD, the results were 71.5%, 69.3%, 40.8%, and 89.2%, respectively, while for the diagnosis of significant fibrosis, the results were 68.3%, 67.5%, 59.9%, and 75.0%, respectively. When compared to those of fibrosis 4 (FIB-4) index, aspartate aminotransferase (AST)-to-platelet ratio index (APRI), and AST-to-alanine aminotransferase (ALT) ratio (AAR), the AUROC for the performance of FIB-6 was higher than that of FIB-4, APRI, and AAR in all fibrosis stages. FIB-6 gave the highest sensitivity and NPV (89.1% and 92.4%) in ruling out cACLD and cirrhosis, as compared to FIB-4 (63.8% and 83.0%), APRI (53.9% and 86.6%), and AAR (47.5% and 82.3%), respectively.

**Conclusions:** The FIB-6 index could be used in ruling out cACLD, fibrosis, and cirrhosis with good reliability.

**Keywords:** CHB, FIB-6, liver cirrhosis, liver fibrosis, non-invasive tests

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## INTRODUCTION

Chronic hepatitis B (CHB) is a major public health burden and a common cause of cirrhosis and complications worldwide. The estimated global prevalence of this viral infection is 3.6%, varying among countries from highly endemic to lower endemicity.[1] The natural history of the disease is variable, with different phases depending on several factors, mainly related to the interaction between the virus and the body's immune system.[2] Liver fibrosis assessment is essential in assessing disease severity and prognosis, anticipating complications, and deciding to provide or defer antiviral therapy.[3,4] Frequent fibrosis assessment in CHB is more critical with the lack of effective curative treatment to eradicate the virus. The treatment is usually reserved for patients with evidence of active and significant disease, mainly those with significant fibrosis. Therefore, long-term monitoring of the disease is required in all hepatitis B patients.

With its challenges and limitations, liver biopsy remains the gold standard tool for fibrosis assessment.[5] Repeated fibrosis assessment using liver biopsy is challenging in clinical practice; therefore, many non-invasive tools (NIT) in the last few decades have been evaluated.

Most of the guidelines recommended using NITs to assess and monitor disease progression in CHB.[6–9] These guidelines recommended liver stiffness measurements (LSM) as the first option; however, LSM is not always accessible in limited-resource settings as well in most general practice clinics. Therefore, serology-based NITs with low cost and easy interpretation are recommended alternatives.

Fibrosis 4 index (FIB-4) and aspartate aminotransferase (AST)-to-platelet ratio index (APRI) are among the most widely used, and were initially developed and validated in chronic hepatitis C (CHC) patients with variable performance and cut-off points.[8,10,11] They have limited variable performance and proposed cut-off values in several studies assessing fibrosis, especially for moderate-severe stages; furthermore, they do not reflect changes in fibrosis stages over time.[11–13] Therefore, there is still an unmet need for the development of netter NIT with high accuracy and easy accessibility in assessing fibrosis.

Recently, our group developed a new score called the fibrosis 6 (FIB-6) index to assess fibrosis in CHC patients. This index was developed using data from a large cohort of biopsy-proven CHC patients using machine learning analysis (depending on Random Forests) and liver biopsy as the gold reference standards. This new index score relies on six readily available clinical and laboratory parameters: age, aspartate, alanine aminotransferases, alkaline phosphatase, albumin (g/dL), and platelet count (/cm$^3$). In both development and validation groups and the cohort from different countries, FIB-6 showed excellent performance in ruling out fibrosis, with higher performance for severe fibrosis stages.[14] In this study we aimed to assess the diagnostic performance of this index in the diagnosis of fibrosis in patients with CHB.

## PATIENTS AND METHODS

### Study design

This was a retrospective observational analysis of data of CHB patients obtained from seven countries (Egypt, Kingdom of Saudi Arabia (KSA), Qatar, Oman, Jordan, Turkey, and Greece).

The laboratory and histological data used for analysis were from treatment-naïve patients, or before initiation of treatment in those who were candidates for treatment. The inclusion criteria were adult patients (age >18 years) with confirmed CHB, which is defined as HBsAg-positive for more than 6 months, with adequate liver biopsy and complete biochemical and hematological data. The exclusion criteria were the coexistence of other liver diseases, such as fatty liver, CHC, autoimmune liver or biliary disease, decompensated cirrhosis, and hepatocellular carcinoma. In addition, patients with hematological or bone-related diseases were excluded.

### Liver biopsy

We used the same process in our work on FIB-6 for CHC patients.[14] The adequate liver biopsy specimens required at least a 20-mm core tissue or 11 portal tracts.[15] Liver histology was scored by experienced pathologists using the METAVIR scoring system. Fibrosis was staged on a 0–4 scale: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis.[16] The classification of fibrosis categories used in this analysis was significant fibrosis (F2–F4 METAVIR), compensated advanced chronic liver disease (cACLD) (F3–F4 METAVIR), and cirrhosis (F4 METAVIR).

### Laboratory testing

Routine hematological and biochemical laboratory parameters were determined using commercially available assays. Blood tests were performed to determine the following: total blood count (automated blood cell analyzer Sysmex XT-1800i; Sysmex Corporation); liver transaminases (ALT/AST);

α-fetoprotein (COBAS INTEGRA 411; Roche); and creatinine, albumin, bilirubin, and glucose/glycated hemoglobin level (COBAS INTEGRA 400 plus; Roche). The laboratory tests were performed at the same time as liver biopsy.

All patients' samples were tested for HBsAg using a commercial enzyme-linked immunoassay (ELISA) kit (Abbott Laboratories, Chicago, IL). All positive patients were further investigated for quantitative hepatitis B virus deoxyribonucleic acid (HBV DNA) by reverse transcription-polymerase chain reaction (RT-PCR) (Applied Biosystems, CA).

## Non-invasive score calculation

In this cohort, we calculated the FIB-6 index in addition to other serum-based scores: FIB-4, APRI, and AST-to-alanine aminotransferase (ALT) ratio (AAR). The calculation was based on the equation used in the original publication of these scores[17–19] using the following equations:

$$\text{APRI} = \frac{\left(\frac{\text{AST level (IU / L)}}{\text{AST upper limit of normal (IU / L)}}\right)}{\text{Platelet count } (\times 10^9 \text{ / L})} \times 100 \quad [17]$$

$$\text{FIB - 4} = \frac{\text{age (years)} \times \text{AST (IU / L)}}{\text{Platelet count}(\times 10^9 \text{ / L}) \times \sqrt{\text{ALT (IU / L)}}} \quad [18]$$

$$\text{AAR} = \frac{\text{AST}\left(\text{IU / L}\right)}{\text{ALT}\left(\text{IU / L}\right)} \quad [19]$$

FIB-6 was calculated using the website: http://fib6.elriah.info/. The development of FIB-6 was fully explained previously in a published paper.[14] It is a machine learning algorithm depending on six parameters (age, AST, ALT, alkaline phosphatase, albumin, and platelet count) together with the upper limit of normal for AST, ALT, and alkaline phosphatase.

## Statistical analysis

For statistical analysis, we used the Statistical Package for Social Sciences (SPSS) version 26, easyROC for receiver operating characteristic (ROC) curve analysis (http:// www. http://biosoft.erciyes.edu.tr/app/easyROC), and OpenEpi version 3.01 for diagnostic test performance evaluation (www.openepi.com).

Quantitative data are expressed as mean ± standard deviation or median and the interquartile range for normally or abnormally distributed data according to normality tests performed using the Kolmogorov-Smirnov test. Qualitative data were expressed as a percentage. Student's *t*-test and Mann-Whitney U-test were performed for the comparison of continuous parameters, whereas the Chi-square test was used for the comparison of qualitative data.

The ROC curves were plotted to measure and compare the performance of different non-invasive parameters in predicting liver fibrosis. The diagnostic performance of the new scoring system was assessed using ROC for different fibrosis stages, and its best cut-off values were chosen to calculate sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), LR+ (likelihood ratio of a positive test), LR- (likelihood ratio of a negative test) accuracy, diagnostic odds ratio (DOR), and area under the receiver operating characteristic curve (AUROC). To select a proper diagnostic test, researchers recommended using the most specific test to confirm (rule-in) diagnosis and the most sensitive test to establish that a disease is unlikely (rule-out).

Three cut-offs were calculated: the optimal cut-off (using Youden Index, (sensitivity + specificity − 1)), rule-in cut-off (resulting in best PPV and specificity), and rule-out cut-off (resulting in best NPV and sensitivity). Then, the performance of these cut-offs was validated. For comparison, conventional cut-offs were applied to rule out severe fibrosis and cirrhosis. For FIB-4, cut-offs that were previously shown to rule out severe fibrosis and cirrhosis were applied (FIB-4 <1.45). Moreover, we used <0.7 for APRI. Similarly, for AAR, we used a cut-off point of 1.

DeLong test was used to compare the difference between two areas under the curve (AUCs).

A two-tailed test of significance was used. $P < 0.05$ was considered significant.

**Table 1: Characteristics of study patients**

| Variable | Result |
|---|---|
| Male/female, *n* (%) | 435 (72.1%)/168 (27.9%) |
| Age (years) | 43±12 |
| BMI (kg/m²) | 28.35±5.67 |
| ALT (IU/L) | 51±82 |
| AST (IU/L) | 90±90 |
| ALP (IU/L) | 85.8±50.7 |
| Albumin (g/L) | 4.3±6.0 |
| Platelets (x10^9/L) | 202±67 |
| Fibrosis stages | |
| - F0 | 103 (17.1%) |
| - F1 | 251 (41.6%) |
| - F2 | 112 (18.6%) |
| - F3 | 58 (9.6%) |
| - F4 | 79 (13.1%) |

Values are the mean±SD (standard deviation). BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; F: fibrosis stage based on Metavir system

## RESULTS

In this study, 603 patients met the criteria, and their data were included for analysis, with a mean age of 43 ± 12 years, in which 435 (72.1%) were males, and the mean ALT level was 51 ± 82 IU/L; Table 1 shows the characteristics of the cohort included. With regard to fibrosis stage categories in this cohort, significant fibrosis was diagnosed in 41%, cACLD in 23%, and cirrhosis in 13% of the patients.

Analysis of FIB-6 performance showed higher FIB-6 score associated with higher rates of F3 and F4. Indeed, F3 and F4 stages were rarely detected in the lowest quartile of FIB-6, whereas they were detected in 81 (54.4%) cases in the highest quartile [Figure 1].

The AUROC of FIB-6 for the discrimination of patients with cirrhosis (F4), cACLD (F3–F4), and significant fibrosis (F2–F4) from those without was 0.854 (95% CI, 0.767–0.881; $P < 0.001$), 0.812 (95% CI, 0.745–0.838; $P < 0.001$), and 0.745 (95% CI, 0.704–0.786; $P < 0.001$), respectively [Table 2].

Using the optimal cut-offs of FIB-6 for the diagnosis of cirrhosis (FIB-6 = 2.3159), cACLD (FIB-6 = 1.8992), and significant fibrosis (FIB-6 = 1.7720) indicated a reliable performance of FIB-6 score. For the diagnosis of cirrhosis, sensitivity was 70.9%, specificity was 84.1%, PPV was 40.3%, and NPV was 95.0%. For the diagnosis of cACLD (F3 and F4), the results were 71.5%, 69.3%, 40.8%, and 89.2%, respectively, while for the diagnosis of significant fibrosis (F2, F3, and F4), the results were 68.3%, 67.5%, 59.9%, and 75.0%, respectively [Table 2]. Analysis using other cut-off points is presented in Supplementary Table 1.

When the performance result of FIB-6 was compared to those of FIB-4, APRI, and AAR, the AUROC for the performance of FIB-6 was higher than that of FIB-4, APRI, and AAR in all fibrosis stages; however, DeLong test was statistically significant for comparison with AAR [Figure 2].

To compare FIB-6 performance with the other scores, we used the rule-out cut-off value for cACLD of FIB-6 (1.5023) and the rule-out values reported in other studies of the other scores. FIB-6 gave the highest sensitivity and NPV (89.1% and 92.4%) in ruling out cACLD and cirrhosis, as compared to FIB-4 (63.8% and 83.0%), APRI (53.9% and 86.6%), and AAR (47.5% and 82.3%), respectively [Table 3].

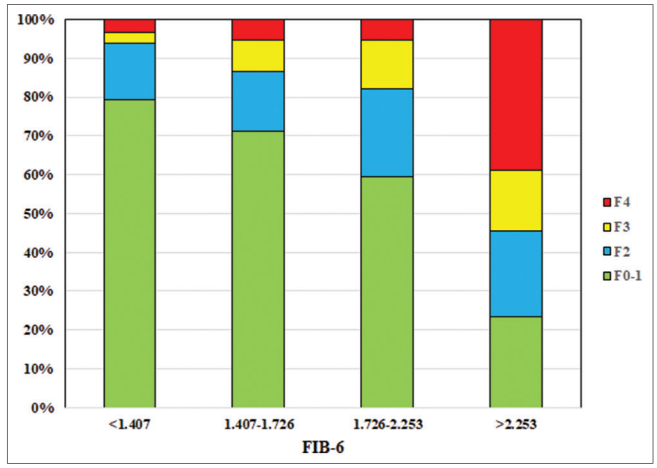



**Figure 1:** Relationship between the FIB-6 and Metavir stage of liver fibrosis in the cohort. FIB-6 data are divided into four quartiles

The results of the analysis of FIB-6 performance in different cohorts from participating countries for different fibrosis stages are shown in Table 4. There is a wide variation in the cohort size from these countries, as the largest cohort was from Egypt. Similar to the observation in the whole cohort, it is notable that the NPVs of FIB-6 were high (90.9–100%), with the highest NPVs for higher degrees of fibrosis with similar trending seen in the whole cohort, with the highest results in more severe fibrosis stages. The superior performance of FIB-6 with high sensitivity and NPV compared to other evaluated scores was also observed and consistent in all sub-analysis of individual country analysis [Supplementary Table 2].

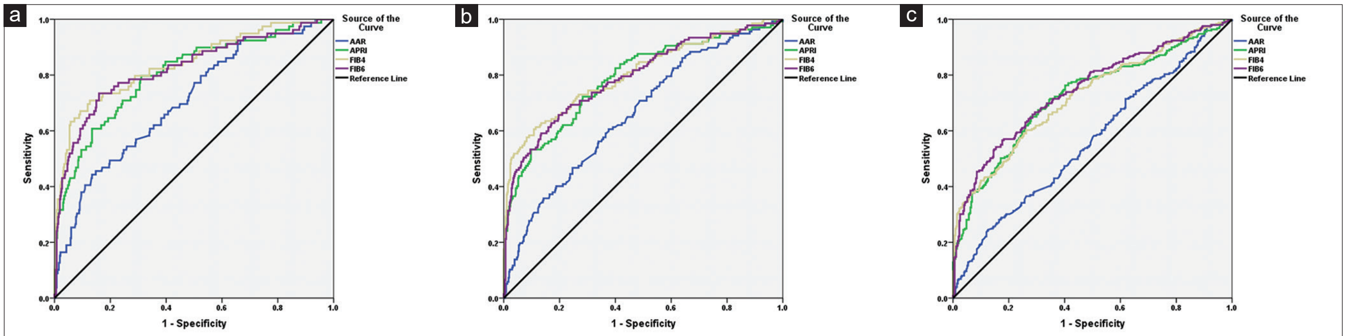
## DISCUSSION

The assessment of liver fibrosis is of paramount importance for clinical decision-making, especially in chronic liver disease with a lack of curative treatment and dynamic disease course, such as CHB, which requires long-term follow-up of the disease, including fibrosis changes over time. All NIT have limitations and suboptimum accuracy, especially in CHB.[10,12,13,20] In this study, we assessed the performance of FIB-6 using liver histology as a reference

**Table 2: Diagnostic performance of FIB-6 optimal cut-offs in all cohorts compared to the liver biopsy results**

| | AUROC | Cut-off | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|---|
| Cirrhosis (F4 vs. F0123) | 0.854 | 2.3159 | 70.9 (60.1-79.8) | 84.1 (80.7-87.0) | 40.3 (32.5-48.6) | 95.0 (92.6-96.7) |
| cACLD (F34 vs. F012) | 0.812 | 1.8992 | 71.5 (63.5-78.4) | 69.3 (65.0-73.4) | 40.8 (34.8-47.2) | 89.2 (85.5-92.0) |
| Significant fibrosis (F234 vs. F01) | 0.745 | 1.7720 | 68.3 (62.3-73.7) | 67.5 (62.5-72.2) | 59.9 (54.1-65.4) | 75.0 (69.9-79.5) |

AUROC, area under the receiver operator characteristic curve; cACLD, compensated advanced chronic liver disease; NPV, negative predictive value; PPV, positive predictive value




| a | FIB-6 | FIB-4 | APRI | AAR |
|---|---|---|---|---|
| AUROC | 0.854 | 0.841 | 0.805 | 0.705 |
| SE | 0.029 | 0.027 | 0.028 | 0.032 |
| 95% CI | 0.797-0.911 | 0.787-0.894 | 0.749-0.861 | 0.644-0.767 |
| P-value | <0.001 | <0.001 | <0.001 | <0.001 |
| DeLong# | | 0.258 | 0.293 | 0.001 |

| b | FIB-6 | FIB-4 | APRI | AAR |
|---|---|---|---|---|
| AUROC | 0.812 | 0.804 | 0.788 | 0.662 |
| SE | 0.024 | 0.024 | 0.024 | 0.026 |
| 95% CI | 0.765-0.858 | 0.758-0.851 | 0.741-0.834 | 0.610-0.713 |
| P-value | <0.001 | <0.001 | <0.001 | <0.001 |
| DeLong# | | 0.425 | 0.837 | <0.001 |

| c | FIB-6 | FIB-4 | APRI | AAR |
|---|---|---|---|---|
| AUROC | 0.745 | 0.723 | 0.723 | 0.564 |
| SE | 0.021 | 0.021 | 0.022 | 0.024 |
| 95% CI | 0.704-0.786 | 0.681-0.765 | 0.681-0.766 | 0.517-0.611 |
| P-value | <0.001 | <0.001 | <0.001 | 0.007 |
| DeLong# | | 0.200 | 0.265 | <0.001 |

**Figure 2:** Receiver operator curve (ROC) analysis of fibrosis 6 (FIB-6) for the diagnosis of (a) cirrhosis (F4 Metavir), (b) compensated advanced chronic liver disease (F3–F4 Metavir), and (c) significant fibrosis (F2–F4 Metavir), compared with fibrosis 4 (FIB-4), aminotransferase-to-platelet ratio index (APRI), and aspartate aminotransferase-to-alanine aminotransferase ratio (AAR) in the derivation dataset. "In any of the three fibrosis stages, FIB-6 performed better than other non-invasive indices (FIB-4, APRI, and AAR) as indicated by higher AUROC." AUROC, area under the receiver operating characteristic curve. #*P*-value of DeLong test comparing FIB-4, APRI, and AAR with FIB-6

in a large multinational cohort of CHB using the cut-off values generated in the original work of the FIB-6 index.

The demographic of our cohort showed that the majority of the patients were male and relatively young, with lower fibrosis stage distribution. This is similar to several studies assessing NITs in CHB.[10,20] However, our cohort has more patients with higher body mass index (BMI), reflecting the increasing prevalence of obesity in the Middle East.[21]

The levels of ALT can affect the result of NIT; therefore, ALT levels should be taken into account when performing or interpreting these NIT. In our cohort, the mean ALT level was <2 ULN, and this favors less bias with ALT flares on NITs causing falsely higher results.[22,23]

In our analysis, the performance of FIB-6 showed excellent discrimination ability for cACLD and cirrhosis and less for lower fibrosis stages, in which the AUROC for discrimination of cirrhosis, cACLD (F3-4), and significant fibrosis from the lower stages was 0.854, 0.812, and 0.745, respectively. This performance is lower than the performance of this test in CHC, which was 0.917, 0.914, and 0.839 for cirrhosis, cACLD, and significant fibrosis, respectively. This finding was consistent in all cohorts from all participating countries. On the other hand, the lower performance of NITs in CHB is consistent in many studies[12,13,24] and is not fully explained. The variable immune activity in CHB and disease natural history, accounting for different degrees of inflammation and related ALT levels may play a part in accuracy and consistency, and render the need for different cut-off levels in CHB with different inflammation and activity levels.[25,26]

We presented in detail the optimum cut-off as this is more relevant to clinical practice. However, our results showed that FIB-6 NPV was higher than PPV in all fibrosis stages, indicating that this score is higher in ruling out rather than ruling in fibrosis; this is similar to the performance of this score in the CHC cohort.[14]

Compared to APRI and FIB-4, this score showed better performance with higher AUROC and performance statistics; this was consistent in all fibrosis stages, making this score potentially superior to these commonly used scores. Furthermore, in addition to the limited diagnostic accuracy of APRI and FIB-4, these tests have also shown less reliable results in long-term assessment of fibrosis regression, as assessed in a large cohort enrolled

**Table 3: Diagnostic performance of FIB-6 rule-out cut-offs for cACLD (F3) or cirrhosis (F4) in all cohorts compared with that of FIB-4, APRI, and AAR**

| | Cut-off | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|
| FIB-6 | 1.5023 | 89.1 (82.7-93.3) | 39.5 (35.2-44.1) | 30.4 (26.1-35.0) | 92.4 (87.9-95.4) |
| FIB-4 | 1.45 | 63.8 (55.6-71.3) | 83.0 (79.4-86.1) | 52.6 (45.2-60.0) | 88.6 (85.3-91.2) |
| APRI | 0.70 | 53.9 (45.7-61.9) | 88.3 (85.1-90.9) | 57.6 (48.1-65.7) | 86.6 (83.3-89.4) |
| AAR | 1.00 | 47.5 (39.5-55.7) | 72.0 (67.8-75.8) | 33.3 (27.2-40.1) | 82.3 (78.4-85.7) |

NPV, negative predictive value; PPV, positive predictive value; FIB-6, fibrosis 6 index; FIB-4, fibrosis 4 index; APRI, aminotransferase-to-platelet ratio index; AAR, aspartate aminotransferase-to-alanine aminotransferase ratio

**Table 4: Diagnostic performance of FIB-6 optimal cut-offs compared to the liver biopsy results in each country**

| | No | Cut-off | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|---|---|
| I-Egypt (324 patients) | | | | | | |
| Cirrhosis (F4 vs. F0123) | 51 | 2.3159 | 58.8 (42.2-71.5) | 87.2 (82.7-90.6) | 46.2 (34.6-58.2) | 91.9 (87.9-94.6) |
| cACLD (F34 vs. F012) | 75 | 1.8992 | 67.1 (55.9-76.6) | 77.0 (71.4-81.8) | 47.2 (38.1-56.6) | 88.4 (83.5-92.0) |
| Significant fibrosis (F234 vs. F01) | 118 | 1.7720 | 64.5 (55.6-72.4) | 74.4 (68.0-79.9) | 60.0 (51.4-68.0) | 77.8 (71.5-83.1) |
| II-KSA (90 patients) | | | | | | |
| Cirrhosis (F4 vs. F0123) | 4 | 2.3159 | 75.0 (30.1-95.4) | 72.3 (61.8-80.8) | 11.5 (4.0-29.0) | 98.4 (91.3-99.7) |
| cACLD (F34 vs. F012) | 10 | 1.8992 | 100.0 (70.1-100.0) | 48.7 (38.0-59.6) | 18.4 (10.0-31.4) | 100.0 (90.8-100.0) |
| Significant fibrosis (F234 vs. F01) | 43 | 1.7720 | 77.5 (62.5-87.7) | 51.1 (37.2-64.7) | 57.4 (44.2-69.7) | 72.7 (55.8-84.9) |
| III-Turkey (86 patients) | | | | | | |
| Cirrhosis (F4 vs. F0123) | 8 | 2.3159 | 100.0 (67.6-100.0) | 85.9 (76.5-91.9) | 42.1 (23.1-63.7) | 100.0 (94.6-100.0) |
| cACLD (F34 vs. F012) | 19 | 1.8992 | 79.0 (56.7-91.5) | 64.2 (52.2-74.6) | 38.5 (24.9-54.1) | 91.5 (80.1-96.6) |
| Significant fibrosis (F234 vs. F01) | 35 | 1.7720 | 74.3 (57.9-85.8) | 51.0 (37.7-64.1) | 51.0 (37.7-64.1) | 74.3 (57.9-85.8) |
| IV-Greece (43 patients) | | | | | | |
| Cirrhosis (F4 vs. F0123) | 6 | 2.3159 | 100.0 (61.0-100.0) | 81.1 (65.8-90.5) | 46.2 (23.2-70.9) | 100.0 (88.7-100.0) |
| cACLD (F34 vs. F012) | 14 | 1.8992 | 57.1 (32.6-78.6) | 55.2 (37.6-71.6) | 38.1 (20.8-59.1) | 72.7 (51.9-86.9) |
| Significant fibrosis (F234 vs. F01) | 22 | 1.7720 | 59.1 (38.7-76.7) | 52.4 (32.4-71.7) | 56.5 (36.8-74.4) | 55.0 (34.2-74.2) |
| V-Oman (22 patients) | | | | | | |
| Cirrhosis (F4 vs. F0123) | 1 | 2.3159 | 100.0 (20.7-100.0) | 85.7 (65.4-95.0) | 25.0 (4.6-69.9) | 100.0 (82.4-100.0) |
| cACLD (F34 vs. F012) | 4 | 1.8992 | 50.0 (15.0-85.0) | 72.2 (49.1-87.5) | 28.6 (8.2-64.1) | 86.7 (62.1-96.3) |
| Significant fibrosis (F234 vs. F01) | 9 | 1.7720 | 55.6 (26.7-81.1) | 84.6 (57.8-95.7) | 71.4 (35.9-97.8) | 73.3 (48.1-89.1) |
| VI-Qatar (20 patients) | | | | | | |
| Cirrhosis (F4 vs. F0123) | 3 | 2.3159 | 100.0 (43.9-100.0) | 88.2 (65.7-96.7) | 60.0 (23.1-88.2) | 100.0 (79.6-100.0) |
| cACLD (F34 vs. F012) | 7 | 1.8992 | 100.0 (64.6-100.0) | 100.0 (77.2-100.0) | 100.0 (64.6-100.0) | 100.0 (77.2-100.0) |
| Significant fibrosis (F234 vs. F01) | 11 | 1.7720 | 72.7 (43.4-90.3) | 77.8 (45.3-93.7) | 80.0 (89.0-94.3) | 70.0 (39.7-89.2) |
| VII-Jordan (18 patients) | | | | | | |
| Cirrhosis (F4 vs. F0123) | 6 | 2.3159 | 83.3 (43.7-97.0) | 83.3 (55.2-95.3) | 71.4 (35.9-91.8) | 90.9 (62.3-98.4) |
| cACLD (F34 vs. F012) | 8 | 1.8992 | 75.0 (40.9-92.9) | 70.0 (39.7-89.2) | 66.7 (35.4-87.9) | 77.8 (45.3-93.7) |
| Significant fibrosis (F234 vs. F01) | 11 | 1.7720 | 81.8 (52.3-94.9) | 100.0 (64.6-100.0) | 100.0 (70.1-100.0) | 77.8 (45.3-93.7) |

NPV, negative predictive value; PPV, positive predictive value; cACLD, compensated advanced chronic liver disease (F3–F4 Metavir)

in registration trials at 5 years.[11] The ability of FIB-6 in long-term assessment for fibrosis regression is to be determined in long-term studies.

Our study has several strengths: First, this study is the first validation of this new novel score in CHB. Second: a large number of patients from a diverse international cohort demonstrated consistent performance of the score, enhancing result reliability. Additionally, this test outperformed other commonly used tests across all fibrosis categories.

However, our study has some limitations: First, the retrospective design is prone to selection bias (however, most of the first score studies are retrospective in design); second, the gold standard reference used, the liver biopsy, has limitations and potential for sampling error, impacting the result; and third, the proportion of lower fibrosis stages in this study is higher than that of cACLD and cirrhosis; however, this is generally the case in most studies assessing NIT.

In conclusion, our study showed that FIB-6 is a reliable, non-invasive, and simple score in assessing fibrosis in CHB using readily available data. The performance results are promising, especially in ruling out cACLD and cirrhosis; a future prospective study will give more validation of this conclusion and the best cut-off value.

## Approval of the research protocol

The study protocol was approved by the Research and Ethics Committee of ELRIAH (OHRP IRB #8819). The protocol and conduct of the study complied with the International Ethical Guidelines for Biomedical Research Involving Human Subjects and its amendments, 2008. The protocol was also approved by the Institutional Research Board of each participating center, as per local regulations.

## Informed consent

The requirement for written informed consent was waived by the ethics committee as this was an analysis of anonymized patient data collected during routine clinical care.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

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**Supplementary Table 1: Diagnostic performance of FIB-6 cut-offs in the CHB cohorts**

| Fibrosis stage | No | clinical use | Cut-off | Sensitivity | Specificity | PPV | NPV | LR + | LR - | Accuracy | DOR |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cirrhosis (F4 vs. F0123) | 79 | Rule-out | 1.7017 | 84.8 (75.3-91.1) | 53.7 (49.5-58.0) | 21.8 (17.5-26.7) | 95.9 (93.0-67.6) | 1.83 (1.81-1.86) | 0.28 (0.24-0.33) | 57.8 (53.8-61.7) | 6.5 (3.4-12.3) |
| | | Optimal | 2.3159 | 70.9 (60.1-79.8) | 84.1 (80.7-87.0) | 40.3 (32.5-48.6) | 95.0 (92.6-96.7) | 4.45 (4.28-4.62) | 0.35 (0.32-0.38) | 82.3 (79.1-85.2) | 12.9 (7.5-22.0) |
| | | Rule-in | 3.1718 | 45.6 (35.1-56.5) | 96.0 (93.9-97.4) | 63.2 (50.2-74.5) | 92.1 (89.5-94.1) | 11.31 (9.65-13.25) | 0.57 (0.54-0.59) | 89.3 (86.6-91.6) | 19.9 (10.7-37.1) |
| cACLD (F34 vs. F012) | 137 | Rule-out | 1.5023 | 89.1 (82.7-93.3) | 39.5 (35.2-44.1) | 30.4 (26.1-35.0) | 92.4 (87.9-95.4) | 1.47 (1.46-1.59) | 0.28 (0.24-0.32) | 50.8 (46.8-54.8) | 5.3 (3.0-9.4) |
| | | Optimal | 1.8992 | 71.5 (63.5-78.4) | 69.3 (65.0-73.4) | 40.8 (34.8-47.2) | 89.2 (85.5-92.0) | 2.33 (2.28-2.38) | 0.41 (0.39-0.43) | 69.8 (66.0-73.4) | 5.7 (3.7-8.6) |
| | | Rule-in | 2.7656 | 44.5 (36.5-52.9) | 95.9 (93.7-97.4) | 76.3 (65.9-84.2) | 85.4 (82.1-88.2) | 10.85 (9.40-12.52) | 0.58 0.56-0.59) | 84.2 (81.0-86.9) | 18.8 (10.6-33.2) |
| Significant fibrosis (F234 vs. F01) | 249 | Rule-out | 1.0722 | 97.6 (94.8-98.9) | 8.5 (6.1-11.9) | 43.1 (39.1-47.2) | 83.3 (68.1-92.1) | 1.07 (1.06-1.07) | 0.28 (0.10-0.79) | 45.5 (41.6-49.5) | 3.8 (1.6-9.2) |
| | | Optimal | 1.7720 | 68.3 (62.3-73.7) | 67.5 (62.5-72.2) | 59.9 (54.1-65.4) | 75.0 (69.9-49.5) | 2.10 (2.06-2.15) | 0.47 (0.46-0.48) | 67.8 (64.0-71.5) | 4.5 (3.2-6.3) |
| | | Rule-in | 2.6284 | 33.3 (27.8-39.4) | 96.0 (93.4-97.6) | 85.6 (77.2-91.2) | 67.0 (66.8-73.5) | 8.35 6.93-10.08) | 0.69 (0.69-0.70) | 70.0 (66.2-73.5) | 12.0 (6.6-21.8) |

cACLD, compensated advanced chronic liver disease; DOR, diagnostic odds ratio; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value

**Supplementary Table 2: Diagnostic performance of FIB-6 rule-out cut-offs for cACLD (F3-F4) in the evaluation cohorts compared with that of FIB-4, APRI, and AAR**

| | Cut-off | Sensitivity | NPV |
|---|---|---|---|
| I-Egypt (324 patients) | | | |
| FIB-6 | 1.5023 | 85.5 (75.9-91.7) | 91.4 (85.3-95.1) |
| FIB-4 | 1.45 | 63.2 (51.9-73.1) | 87.3 (82.3-91.1) |
| APRI | 0.70 | 47.4 (36.5-58.5) | 84.7 (79.8-88.5) |
| AAR | 1.00 | 51.3 (40.3-62.2) | 80.4 (74.2-85.5) |
| II-KSA (90 patients) | | | |
| FIB-6 | 1.5023 | 100 (70.1-100.0) | 100.0 (82.4-100.0) |
| FIB-4 | 1.45 | 60.0 (31.3-83.2) | 95.1 (88.1-98.1) |
| APRI | 0.70 | 80.0 (49.0-94.3) | 97.2 (90.4-99.23) |
| AAR | 1.00 | 44.4 (18.9-73.3) | 94.1 (86.8-97.4) |
| III-Turkey (86 patients) | | | |
| FIB-6 | 1.5023 | 89.5 (68.6-97.1) | 90.9 (72.2-97.5) |
| FIB-4 | 1.45 | 77.3 (56.6-89.9) | 92.9 (84.3-96.9) |
| APRI | 0.70 | 72.7 (51.9-86.9) | 91.7 (83.0-96.1) |
| AAR | 1.00 | 45.5 (26.9-65.3) | 82.9 (72.4-89.9) |
| IV-Greece (43 patients) | | | |
| FIB-6 | 1.5023 | 85.7 (60.1-96.0) | 85.7 (60.1-96.0) |
| FIB-4 | 1.45 | 42.9 (21.4-67.4) | 70.4 (52.5-84.2) |
| APRI | 0.70 | 28.6 (11.7-54.7) | 71.4 (54.9-83.7) |
| AAR | 1.00 | 28.6 (11.7-54.7) | 71.4 (54.9-83.7) |
| V-Oman (22 patients) | | | |
| FIB-6 | 1.5023 | 100.0 (51.0-100.0) | 100.0 (67.6-100.0) |
| FIB-4 | 1.45 | 25.0 (4.6-69.9) | 85.7 (65.4-95.0) |
| APRI | 0.70 | 25.0 (4.6-69.9) | 85.0 (64.0-94.8) |
| AAR | 1.00 | 25.0 (4.6-69.9) | 80.0 (54.8-93.0) |
| VI-Qatar (20 patients) | | | |
| FIB-6 | 1.5023 | 100.0 (64.6-100.0) | 100.0 (51.0-100.0) |
| FIB-4 | 1.45 | 85.7 (48.7-97.4) | 92.9 (68.5-98.7) |
| APRI | 0.70 | 71.4 (35.9-91.8) | 85.7 (60.1-96.0) |
| AAR | 1.00 | 42.9 (15.8-75.0) | 69.2 (42.4-87.3) |
| VII-Jordan (18 patients) | | | |
| FIB-6 | 1.5023 | 100.0 (67.6-100.0) | 100.0 (51.0-100.0) |
| FIB-4 | 1.45 | 75.0 (40.9-92.9) | 81.8 (52.3-94.9) |
| APRI | 0.70 | 75.0 (40.9-92.9) | 83.3 (55.2-95.3) |
| AAR | 1.00 | 75.0 (40.9-92.9) | 81.8 (52.3-94.9) |

NPV, negative predictive value; cACLD, compensated advanced chronic liver disease (F3–F4 Metavir); FIB-6, fibrosis 6 index; FIB-4, fibrosis 4 index; APRI, aminotransferase-to-platelet ratio index; AAR, aspartate aminotransferase-to-alanine aminotransferase ratio