CLINICAL RESEARCH

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The Silent Face of Chronic Hepatitis B: Biopsy-Supported Fibrosis Detection and the Reliability of Non-Invasive Scores (FIB-4, APRI) in Inactive, Gray Zone, and Immune-Tolerant Cases

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEFG **Mehmet Kasım Aydın**

Department of Gastroenterology, Faculty of Medicine, Mersin University, Mersin, Türkiye

Corresponding Author: Mehmet Kasım Aydın, e-mail: mkasim.aydin3321@gmail.com
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Background: This study sought to evaluate the diagnostic performance of the non-invasive fibrosis scores Fibrosis-4 Index (FIB-4) and Aspartate Aminotransferase-to-Platelet Ratio Index (APRI) in predicting liver fibrosis among patients with chronic hepatitis B (CHB) in immune-tolerant, inactive, and gray zone phases.

Material/Methods: This retrospective cross-sectional study included 230 patients with CHB, as determined by laboratory and clinical criteria, and who underwent liver biopsy. Patients were grouped based on FIB-4 and APRI fibrosis scores of <3 and ≥3. The FIB-4 and APRI scores were calculated, and their diagnostic accuracy was assessed, using receiver operating characteristic (ROC) curve analysis.

Results: The mean age of the patients was 44.4±12.2 years, and 53.9% were female. A total of 37.4% (86/230) of the patients met the criteria for treatment. Both FIB-4 (1.53±0.90 vs 0.91±0.55, *P*=0.003) and APRI (0.44±0.23 vs 0.29±0.15, *P*=0.001) scores were significantly higher in patients with fibrosis score ≥3. The area under the curve (AUC) was 0.70 (cut-off >1.06) for FIB-4 and 0.68 (cut-off >0.38) for APRI. Both scores had a negative predictive value of 87%. The difference between AUC values was not statistically significant (*P*=0.80).

Conclusions: FIB-4 and APRI are helpful non-invasive tools for ruling out advanced fibrosis in CHB patients. However, due to their limited diagnostic power, they should be considered as supportive tools rather than definitive alternatives to liver biopsy.

Keywords: Chronic Disease • Fibrosis • Hepatitis B • Statistics

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Introduction

Chronic hepatitis B (CHB) infection remains one of the leading causes of liver cirrhosis and hepatocellular carcinoma worldwide. Its natural course consists of 4 phases: immune-tolerant, immune-active, inactive carrier, and reactivation [1,2].

The immune-tolerant phase is marked by elevated hepatitis B virus (HBV) DNA and positive Hepatitis B Virus Early Antigen (HBeAg), while alanine aminotransferase (ALT) remains normal. In contrast, the inactive phase is defined by HBeAg negativity, low HBV DNA levels (<2000 IU/mL), and normal ALT values [2,3].

However, a subgroup of patients present with HBeAg negativity, HBV DNA ≥2000 IU/mL, and persistently normal ALT levels; test results that do not fit clearly into the standard classification system. This group is often referred to as the "gray zone." Although these patients may be classified as inactive carriers, repeated liver biopsies and non-invasive assessments have revealed that some of them may experience subclinical but progressive hepatic injury [4,5].

These patient groups are generally followed without antiviral treatment, and liver biopsy is rarely performed. However, recent studies have demonstrated that these "silent" phases may not be as histologically benign as previously thought [6,7]. In particular, patients who have remained in the immune-tolerant phase for an extended period or those with potential for immune transition due to aging may develop silent fibrosis.

Although liver biopsy is considered the gold standard for assessing liver fibrosis, its invasive nature, risk of complications, and limited patient acceptance restrict its routine use. Consequently, non-invasive biochemical scoring systems such as Fibrosis-4 Index (FIB-4) and Aspartate Aminotransferase-to-Platelet Ratio Index (APRI) have emerged as alternative diagnostic tools, especially in cases where biopsy is not feasible [3,6,8].

The diagnostic value of non-invasive scores in silent phases of HBV infection remains unclear. This study aims to assess the association between histologically confirmed fibrosis stages (F<3 vs F ≥3) and FIB-4 and APRI scores in patients with immune-tolerant, inactive carrier, or gray zone status, and to evaluate their usefulness in detecting significant fibrosis.

Material and Methods

This retrospective cross-sectional study included patients followed at Mersin University Hospital between January 2009 and December 2016. A total of 230 patients with chronic hepatitis B (CHB) who had undergone liver biopsy were included. All patients had documented HBsAg positivity lasting at least 1 year and were classified as being in the immune-tolerant, inactive, or indeterminate (gray zone) phases based on clinical and laboratory criteria. Demographic characteristics (age, sex), laboratory data, HBV DNA levels, and liver biopsy results were retrospectively reviewed.

Inclusion Criteria

1. HBeAg positive with normal ALT levels → Immune-tolerant phase;
2. HBeAg negative, HBV DNA <2000 IU/mL with normal ALT levels → Inactive phase;
3. HBeAg negative, HBV DNA > 2000 IU/mL with normal ALT levels → HBeAg-negative chronic HBV infection (gray zone);
4. Availability of liver biopsy;
5. Patients followed in the same phase (immune-tolerant, inactive carrier, or gray zone) for at least 1 year.

Exclusion Criteria

1. Advanced alcohol consumption
2. Coinfection with hepatitis C virus or human immunodeficiency virus
3. Presence of autoimmune diseases, hemochromatosis, or Wilson's disease
4. Non-alcoholic steatohepatitis (NASH)
5. Immune-active CHB

Histopathological Evaluation

Liver biopsy samples from the study participants were assessed using the histological activity index (HAI). In this scoring system, liver fibrosis severity is rated on a scale ranging from 0 to 6. According to the fibrosis scores obtained, patients were categorized into 2 groups: those with mild or no significant fibrosis (score <3) and those with moderate to advanced fibrosis (score ≥3).

Since the primary objective of the study was to evaluate the diagnostic performance of FIB-4 and APRI scores in predicting histological fibrosis, patient subgroup analyses were classified directly according to liver fibrosis stages rather than clinical phases. This approach was adopted because non-invasive scoring systems such as FIB-4 and APRI primarily aim to estimate the degree of histological fibrosis. Clinical phase classifications are mainly based on virological and biochemical parameters, which may not always correlate directly with fibrosis severity. Therefore, to more accurately assess the true diagnostic performance of these scoring systems, grouping was performed according to fibrosis scores.

Biochemical data for the same patients were used to calculate APRI [9] and FIB-4 [10] scores. The upper reference limit, also known as upper limit of normal (ULN), for ALT and aspartate aminotransferase (AST) was accepted as 32 IU/L, based on the reference ranges used in our hospital laboratory.

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The formulas used were as follows:
APRI=(AST/ULN AST)×100]/platelet count (PLT)
FIB-4=(Age×AST)/[PLT×√ALT]

The obtained FIB-4 and APRI scores were compared with histological fibrosis stages determined according to the HAI system.

Ethical Approval

This retrospective study was approved by the Clinical Research Ethics Committee of Mersin University Faculty of Medicine (Approval Date: June 25, 2025; Approval No: 722).

Statistical Analysis

Continuous variables were expressed as mean±standard deviation with minimum and maximum values, while categorical variables were reported as numbers (n) and percentages (%). Group comparisons for continuous variables were performed using *t*-test.

Receiver operating characteristic (ROC) curve analysis was used to determine optimal cut-off values for FIB-4 and APRI scores. Area under the curve (AUC) values with 95% confidence intervals were calculated. Sensitivity, specificity, and likelihood ratios were used to assess diagnostic performance. ROC curves were also compared using pairwise analysis.

A *P* value <0.05 was considered statistically significant. Analyses were conducted using SPSS v25.0 (IBM Corp., NY, USA) and MedCalc software.

Results

A total of 230 patients in the inactive, immune-tolerant, and gray zone phases were included in this retrospective cross-sectional study. The mean age of the patients was 44.4±12.2 years, with 46.1% (n=106) being male and 53.9% (n=124) female. The need for treatment was present in 37.4% (n=86) of the patients (**Table 1**).

The mean serum ALT level was 21.05±6.07 U/L, AST was 22.08±8.76 U/L, and PLT was 236.66±61.99 (×10^3/μL). The mean HAI score was 4.9±2.2, and the mean fibrosis score was 2.1±0.89. The average FIB-4 and APRI values were 1.04±0.80 and 0.32±0.18, respectively (**Table 1**).

When compared according to fibrosis stage, patients with a fibrosis score ≥3 had significantly higher FIB-4 (1.53±0.90) and APRI (0.44±0.23) values than those with a fibrosis score <3 (*P*=0.003 for FIB-4, *P*=0.001 for APRI) (**Table 2**).

Table 1. Descriptive characteristics of the study population (n=230).

Characteristics	Mean±SD/n	Min-Max
Age	44.4±12.2	18-73
	n	%
Sex		
Male	106	46.1
Female	124	53.9
Treatment requirement		
No	144	62.6
Yes	86	37.4
PT(sec)	13.11±1.65	9.5-16.40
GGT	20.01±12.51	1.74-97.4
INR	1.04±0.11	0.80-1.91
AST	22.08±8.76	2-91.78
ALT	21.05±6.07	7.73-32
PLT	236.66±61.99	100-412
HBsAG	2998.75±573.73	72-4706
HAI	4.9±2.2	1-16
Fibrosis Score	2.1±0.89	0-5
FIB-4	1.04±0.80	0.06-5.98
APRI	0.32±0.18	0.03-1.54

PT – prothrombin time (seconds); GGT – gamma-glutamyl transferase; INR – international normalized ratio; AST – aspartate aminotransferase; ALT – alanine aminotransferase; PLT – platelet count; HBsAg – hepatitis B surface antigen; HAI – histological activity index; APRI – Aspartate Aminotransferase-to-Platelet Ratio Index; FIB-4 – Fibrosis-4 Index.

In the ROC curve analysis, FIB-4 demonstrated an AUC of 0.70 (*P*=0.001) with an optimal cut-off value above 1.06. At this threshold, sensitivity was 70%, specificity 75%, positive predictive value (PPV) 66%, and negative predictive value (NPV) 87%. For APRI, the AUC was 0.68 (*P*=0.001), with a cut-off set at >0.38, yielding a sensitivity of 65%, specificity of 86%, PPV of 66%, and NPV of 87% (see **Table 3 and Figure 1**).

When the diagnostic performances of FIB-4 and APRI were compared, the difference between the ROC curves was not statistically significant (AUC difference: 0.01; 95% CI: -0.07 to 0.09; *P*=0.80) (**Table 4**).

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Table 2. Comparison of FIB-4 and APRI scores according to fibrosis stage in the patients (n=230).

	Fibrosis score <3 n=184	Fibrosis score ≥3 n=46	*P* value
	Mean±SD	Mean±SD	
FIB-4	0.91±0.55	1.53±0.90	0.003
APRI	0.29±0.15	0.44±0.23	0.001

Based on *t*-test, a *P* value less than 0.05 was considered statistically significant. APRI – Aspartate Aminotransferase-to-Platelet Ratio Index; FIB-4 – Fibrosis-4 Index.

Table 3. Cut-off values for FIB-4 and APRI in predicting significant fibrosis (F ≥3).

	AUC	Cut-off	SEN%	SPE%	P value	PPV%	NPV%
FIB-4	0.70	>1.06	70	75	0.001	76	87
APRI	0.68	>0.38	65	86	0.001	66	87

AUC – area under the curve; APRI – Aspartate Aminotransferase-to-Platelet Ratio Index; FIB-4 – Fibrosis-4 Index; NPV – negative predictive value; PPV – positive predictive value; SEN – sensitivity; SPE – specificity.

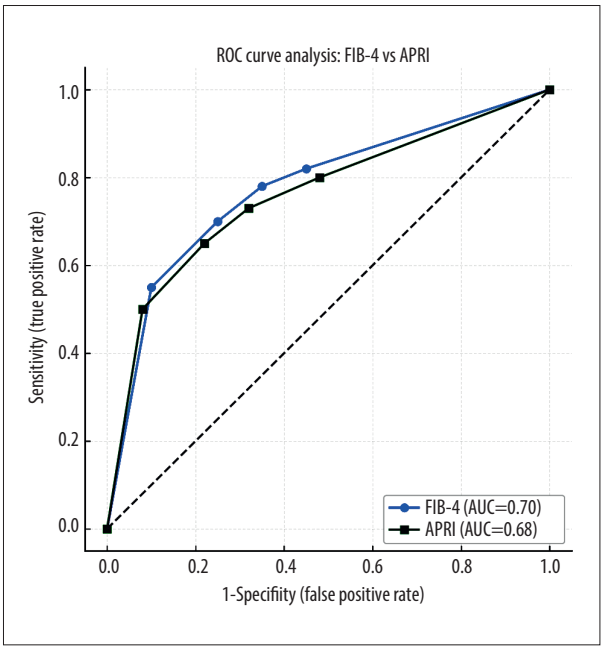

Figure 1. FIB-4 vs APRI ROC Curve. APRI – Aspartate Aminotransferase-to-Platelet Ratio Index; FIB-4 – Fibrosis-4 Index; ROC – receiver operating characteristic.

Discussion

In this study, the efficacy of FIB-4 and APRI scores in the non-invasive prediction of liver fibrosis was evaluated in patients with CHB in the inactive phase, immune-tolerant phase, and gray zone. Our findings demonstrated that both scoring systems were significantly elevated in patients with fibrosis scores ≥3.

Table 4. Pairwise comparison of ROC curves for predicting fibrosis score ≥3 (FIB-4 vs APRI).

Difference between areas	Difference between areas (95% confidence interval)	*P* value
FIB-4-APRI	0.01 (-0.07 to 0.09)	0.80

APRI – Aspartate Aminotransferase-to-Platelet Ratio Index; FIB-4 – Fibrosis-4 Index; ROC – receiver operating characteristic.

In particular, their high negative predictive values (87%) suggest clinical utility in excluding advanced-stage fibrosis [11,12].

However, the AUC values obtained from ROC analysis – 0.70 for FIB-4 and 0.68 for APRI – indicate limited diagnostic power of these tests. Thus, rather than replacing liver biopsy, these scores should be used as supportive tools in the clinical decision-making process [13]. Notably, AUC values below 0.70 imply that these tests may be insufficient as discriminative diagnostic tools in patients with mild to moderate fibrosis.

The significantly higher FIB-4 and APRI scores observed in the advanced fibrosis group can be attributed to the well-established relationship between the parameters included in these scores – such as ALT, AST, and platelet counts – and liver injury and fibrosis [14]. This finding supports the biological rationale underlying these scoring systems.

In our study, cut-off values of >1.06 for FIB-4 and >0.38 for APRI were found to be significant for predicting advanced fibrosis. However, the literature reports variability in these thresholds across different patient populations. For instance, some

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studies have suggested higher cut-offs, such as >1.45 for FIB-4 and >0.5 for APRI [9,10]. These differences may be due to multiple factors, including the ethnic background of the studied population, HBV genotype, degree of inflammatory activity, and differences in fibrosis assessment methods.

One notable finding of our study is that both FIB-4 and APRI tests exhibit high negative predictive values (87%) in excluding advanced fibrosis. This suggests that patients with low FIB-4 or APRI scores have a low probability of developing advanced-stage fibrosis, which in turn raises the possibility of reducing the need for liver biopsy in these cases. In particular, since patients in the immune-tolerant and inactive phases generally exhibit minimal inflammation and fibrotic activity, the potential of these noninvasive markers to decrease biopsy requirements is further strengthened [15].

On the other hand, the limited AUC values obtained from ROC analysis for both tests (0.70 for FIB-4 and 0.68 for APRI) indicate that these scores are insufficient for definitively diagnosing advanced fibrosis. The literature emphasizes that an ideal noninvasive test should have an AUC above 0.80 [13,16]. From this perspective, while FIB-4 and APRI scores may aid in excluding advanced fibrosis, it is clear that they are not sufficient alone for diagnostic certainty.

Cut-off values most commonly recommended in the literature for FIB-4 generally range between 1.45 and 3.25. However, in our study, the optimal threshold for diagnostic accuracy was identified as >1.06 [14,15]. Similarly, although different studies report APRI cut-off values between 0.5 and 1.0, the highest sensitivity and specificity combination in our cohort was achieved at a threshold of >0.38. This finding highlights the importance of population-specific cut-off determination.

When comparing the ROC curves of FIB-4 and APRI scores, no statistically significant difference was found. This suggests that both tests offer comparable diagnostic value in clinical use. Nonetheless, FIB-4's slightly higher AUC and sensitivity may make it preferable for excluding fibrosis. Conversely, APRI's higher specificity suggests that a positive test result may be a stronger indicator of advanced fibrosis. In this context, evaluating FIB-4 and APRI complementarily may provide a more balanced and reliable approach to patient management.

Although our study showed that FIB-4 and APRI scores possess significant diagnostic power for predicting advanced fibrosis (fibrosis score ≥3), the ROC-derived AUC values below 0.70 reveal limited discriminative capacity, especially in patients with mild to moderate fibrosis. This limitation is not unique to our study but is similarly emphasized in the literature.

For instance, Xiao et al (2016) reported that while FIB-4 and APRI scores exhibited acceptable sensitivity and specificity in patients with advanced fibrosis, false-negative rates markedly increased in those with mild and moderate fibrosis [8]. Similarly, Uçar et al (2013) demonstrated that both APRI and FIB-4 scores had higher diagnostic accuracy in patients with significant fibrosis compared with those with no or minimal fibrosis [17].

Limitations

This study has several limitations. Its retrospective, cross-sectional design limits causal inference and may be affected by missing data. The relatively small sample size, especially in the advanced fibrosis subgroup, restricts the generalizability of the findings. Inclusion was limited to biopsy-confirmed cases, which may not fully represent real-world clinical use of noninvasive tests. Variability in laboratory test timing and standardization may have influenced results. Finally, as a single-center study from a specific geographic region, the applicability of findings to other populations is limited. Prospective multicenter studies with larger cohorts are needed.

Conclusions

FIB-4 and APRI scores demonstrated utility in predicting advanced fibrosis (F ≥3) among CHB patients in immune-tolerant, inactive, and gray zone phases. Both tests exhibited high negative predictive values and were effective in excluding advanced fibrosis. However, AUC values below 0.80 indicate limited accuracy in mild to moderate fibrosis. Therefore, FIB-4 and APRI should be used as complementary tools alongside clinical and laboratory data to guide biopsy decisions.

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Declaration of Figures' Authenticity

All figures submitted have been created by the author who confirms that the images are original with no duplication and have not been previously published in whole or in part.

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