Published Online: 2025 April 19

Research Article

Non-invasive Assessment of Advanced Fibrosis and Steatosis in Chronic Hepatitis B Patients with Comorbid NAFLD

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Received: 20 November, 2024; **Revised:** 3 April, 2025; **Accepted:** 9 April, 2025

Abstract

Background: The co-occurrence of chronic Hepatitis B (CHB) and non-alcoholic fatty liver disease (NAFLD) has become increasingly prevalent in clinical practice. However, reliable non-invasive serological markers that can accurately diagnose hepatic fibrosis and steatosis in patients affected by these dual etiologies remain deficient.

Methods: A cross-sectional study was performed on 99 CHB + NAFLD patients who underwent liver biopsy. Based on histopathological findings, patients were categorized into $S \geq 3$ and $S \leq 2$ groups for fibrosis and $F \geq 2$ and $F < 2$ groups for steatosis. Correlation analysis was conducted using Spearman's method. The diagnostic efficacy of relevant indicators for advanced fibrosis and steatosis was evaluated by receiver operating characteristic (ROC) curves, and the performance of different ROC curves was further compared via the DeLong test.

Results: Data analysis at baseline showed that the mean age of all patients was 37.10 years, and 87.9% (87 of 99) were male. Patients with inflammation grade ≤ 2, steatosis grade ≥ 2, fibrosis stage ≥ 3, and normal ALT levels were about 99.0% (98 of 99), 30.3% (30 of 99), 36.4% (36 of 99), and 25.3% (25 of 99), respectively. Comparative analysis revealed that the $S \geq 3$ group showed significantly higher age, AFP, FIB-4, and Forns Index levels compared to the $S \leq 2$ group, while UA levels were significantly lower (all $P < 0.05$). In patients with CHB + NAFLD, fibrosis staging demonstrated significant positive correlations with age, AFP, FIB-4, and Forns Index, while showing an inverse association with UA levels (all $P < 0.05$). Moreover, steatosis grading was positively associated with WBC and GLU, but negatively correlated with LN (all $P < 0.05$). The individual biomarkers demonstrated low diagnostic accuracy for advanced fibrosis and steatosis in CHB + NAFLD patients, with AUC values ranging from 0.600 to 0.680. However, after constructing multivariate models based on their P-values, the diagnostic performance improved substantially, yielding AUC values of 0.750 to 0.850. Both AUAWPGHL and AUAFFWPAGSGHL showed excellent sensitivity for advanced fibrosis (100% and 91.7%, respectively), without significant difference observed in their ROC curve performance ($P > 0.05$). In addition, WGLMA and WGLMAAH exhibited high specificities in diagnosing $F \geq 2$ steatosis (94.1% and 91.2%, respectively), with WGLMAAH demonstrating significantly superior ROC curve performance ($P < 0.05$).

Conclusions: AUAWPGHL was the optimal biomarker for detecting advanced fibrosis ($S \geq 3$), while WGLMAAH demonstrated superior performance in ruling out significant steatosis ($F \geq 2$) in CHB + NAFLD patients.

Keywords: Non-invasive Diagnosis, Advanced Fibrosis, Steatosis, Chronic Hepatitis B, Non-alcoholic Fatty Liver Disease

1. Background

Chronic Hepatitis B (CHB) and non-alcoholic fatty liver disease (NAFLD) are both liver illnesses that can disrupt liver function, causing severe liver complications and posing serious threats to human life (1). Due to urbanization, along with improvements in living standards and lifestyle changes, CHB + NAFLD is increasingly common in clinical practice. The global prevalence of hepatic steatosis in patients with Hepatitis B virus (HBV) infection has been reported to range from 14% to 70% (2, 3). Although HBV infection and

How to Cite: Zhou Z, Chen F, Zou R, Li Q, Xu X. Non-invasive Assessment of Advanced Fibrosis and Steatosis in Chronic Hepatitis B Patients with Comorbid NAFLD. Hepat Mon. 2025; 25 (1): e158104. https://doi.org/10.5812/hepatmon-158104.

hepatic steatosis can have a dual impact on the liver, whether they synergistically contribute to disease progression remains unclear. Notably, patients with concomitant NAFLD may have a higher clearance of Hepatitis B surface antigen (HBsAg) and HBV replication inhibition, leading to a higher probability of functional cure (4, 5). Conversely, some studies have suggested that NAFLD is an independent risk factor for fibrosis in CHB patients, especially those with severe steatosis accelerating hepatic fibrosis progression (6, 7).

Fibrosis is a common clinicopathologic characteristic in CHB + NAFLD patients. According to research, CHB + NAFLD patients with significant fibrosis/cirrhosis have a significantly increased risk of adverse liver outcomes (8, 9). Furthermore, advanced fibrosis is a vital predictor of antiviral therapy response and clinical prognosis in CHB patients (10). Therefore, besides aiding in the early identification of high-risk patients, accurate evaluation of the extent of advanced fibrosis and steatosis in CHB + NAFLD patients could help in developing appropriate therapeutic regimens for timely disease progression prevention.

Although liver biopsy is currently the gold standard for grading and staging liver histopathological severity, it cannot be employed as a routine screening tool due to its invasive nature and high risk of bleeding, among other biopsy-related complications. Consequently, the World Health Organization (WHO) recommends the use of serum biomarkers and transient elastography (TE) in assessing liver fibrosis in CHB patients (11). These approaches offer the advantages of being non-invasive and easy to perform. Serological indicators commonly used to monitor hepatic fibrosis include the aspartate aminotransferase (AST)-to-platelet (PLT) Ratio Index (APRI), the AST-to-alanine aminotransferase (ALT) ratio (AAR), Fibrosis Index based on the four factors (FIB-4), the glutamyl transpeptidase (GGT)-to-PLT ratio (GPR), S-Index, Forns Index, NAFLD fibrosis score (NFS), and the red blood cell distribution width-to-PLT ratio (RPR) (12, 13). Although these scoring systems have been validated in patients with chronic Hepatitis C (CHC), CHB, and NAFLD, it is noteworthy that their relevant parametric factors function differently in the context of dual etiology, resulting in their use not being as effective as expected (14, 15). Furthermore, the accuracy of TE in assessing hepatic fibrosis could be affected in patients with comorbid steatosis and obesity (16).

2. Objectives

Given the limitations of the aforementioned methods in diagnosing CHB + NAFLD, developing novel non-invasive diagnostic markers more suitable for assessing the degree of hepatic fibrosis and steatosis in CHB + NAFLD patients is imperative. Therefore, we retrospectively collected clinical data from patients diagnosed with CHB + NAFLD-associated hepatic fibrosis and steatosis via pathological examination of liver biopsy and analyzed the correlation of hepatic fibrosis/steatosis with single/multifactorial combination indicators and existing serological diagnostic models. In addition, we evaluated the indicators' diagnostic performance and established a non-invasive diagnostic model that could be used to predict advanced fibrosis and moderate-to-severe steatosis, thus providing a strong diagnostic basis for clinical evaluation.

3. Methods

3.1. Patients

This retrospective study involved 99 CHB + NAFLD patients who were admitted to Shenzhen Third People's Hospital between January 2017 and December 2020. The inclusion criteria were: (1) Patients with HBsAg positivity for > 6 months; and (2) patients with liver biopsy histology consistent with pathological changes of fatty liver disease. Patients with complications including other viral liver diseases, alcoholic liver disease, autoimmune liver disease, drug-induced Hepatitis, human immunodeficiency virus (HIV) infection, decompensated cirrhosis, malignancy, and pregnancy were excluded. All patients provided informed consent, and our ethics committee approved the study protocol on December 30, 2018 (approval No. 2018-014).

3.2. Liver Histopathology

Following ultrasonic localization to determine the puncture site and needle depth, liver tissue samples (1.0 - 2.0 cm in length) were obtained using a 16G puncture needle and fixed in a 10% formaldehyde solution. Subsequently, serial pathology sections were prepared and subjected to hematoxylin-eosin (HE) and fiber staining to establish the degree of inflammation, steatosis, and fibrosis. Two senior physicians examined

the stained sections separately. Inflammation grading (G1 - G4) and fibrosis staging (S1 - S4) were performed using the Scheuer scoring system, with S ≥ 3 defined as advanced fibrosis and F ≥ 2 as moderate-to-severe steatosis involving > 20% of the cellular area.

3.3. Research Methods

The following patients' clinical data were obtained from the hospital's electronic medical record system: (1) Basic information (gender, age, height, and weight); (2) clinical grading and staging information (degree of fibrosis, steatosis, and inflammation); (3) routine blood tests [white blood cell (WBC) count, PLT count, and mean PLT volume (MPV)]; (4) Hepatic Function Index [ALT, AST, GGT, albumin (ALB), and alkaline phosphatase (ALP)]; (5) metabolism-related markers [glucose (GLU), cholesterol (CHOL), triglyceride (TG), total bilirubin (TB), direct bilirubin (DB), and uric acid (UA)]; (6) tumor markers [alpha fetoprotein (AFP)]; (7) four tests for liver fibrosis [procollagen III (PIIIP), hyaluronidase (HA), laminin (LN), and type IV collagen (CIV)]; and (8) HBV markers [HBV deoxyribonucleic acid (DNA) and HBsAg].

3.4. Calculation of Non-invasive Diagnostic Markers

Herein, AAR [AAR = [AST(U/L)]/ ALT(U/L)], APRI [APRI score = [AST(U/L)/ULN]/PLT(10^9/L) × 100], FIB-4 [FIB-4 score = [age (years) × AST(U/L)]/[PLT(10^9/L) × ALT(U/L)$^{1/2}$], GPR [GPR score = [GGT(U/L)/ULN)/ PLT(10^9/L) × 100], Forns Index [Forns Index = 7.811 - 3.131 × ln[PLT(10^9/L)] + 0.781 × ln[GGT (U/L)] + 3.467 × ln[age(years)] - 0.014 × CHOL(mg/dL)], S-Index [S-Index = 1000*GGT(U/L)/ [PLT(10^9/L) × ALB(g/l)^2], and NFS [NFS = -1.675 + 0.037 × age (years) + 0.094 × Body Mass Index (BMI; kg/m^2) + 1.13 × impaired fasting glucose/diabetes (yes = 1, no = 0) + 0.99 × AST (U/L)/ALT(U/L) - 0.013 × PLT(10^9/L) - 0.66 × ALB(g/dL)] scores were determined. The diagnostic criteria for diabetes mellitus (DM) were: (1) A history of DM; or (2) a fasting glucose value ≥ 7.0 mmol/L; or (3) glucose levels ≥ 11.1 mmol/L in venous plasma at 2 h on a glucose tolerance test. Note: Upper limits of normal (ULN) of AST= 40 U/L; and ULN of GGT= 45 U/L.

3.5. Statistical Analysis

SPSS 26.0 and MedCalc 20.0 statistical software were used for statistical analysis. Normally distributed quantitative data were expressed as mean ± standard deviation (x ± s), and intergroup comparisons were performed using independent *t*-tests. Non-normally distributed quantitative data were presented as medians [interquartile ranges (IQR; P25 - P75)] and intergroup comparisons were performed using the Wilcoxon rank sum test. Qualitative data were expressed as relative numbers, with intergroup comparisons performed using the χ^2 test. The correlation between bivariate variables was assessed using Spearman's rank correlation method. Variables with varying P-values in the univariate analysis were included in the multivariate logistic regression analysis, and a forward likelihood ratio stepwise regression method was used to construct the risk prediction model. The diagnostic performance of the relative indicators was evaluated using receiver operating characteristic (ROC) curve analysis. Diagnostic accuracy was evaluated by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The optimal cut-off value was determined using Youden's Index, which represents the optimal combination of specificity and sensitivity. The DeLong test was used to compare the performance of different ROC curves. All tests were two-tailed, and results or differences with $P < 0.05$ were considered statistically significant.

4. Results

4.1. Patient Baseline Characteristics

Among the included CHB + NAFLD patients (mean age = 37.10 years), 87.9% were male, 99.0% had an inflammation grade ≤ 2, 30.3% had a steatosis grade ≥ 2, 36.4% had a fibrosis stage ≥ 3, 25.3% had normal ALT levels, 60.9% were HBeAg positive, and 59.1% had a BMI ≥ 25 kg/m^2. A comparative analysis of the clinical data of patients with different fibrosis stages at baseline (Table 1) revealed statistically significant differences in age, UA, AFP, FIB-4, and Forns Index between the two groups (all $P < 0.05$), with the mean age, AFP level, FIB-4, and Forns Index of patients in the S ≥ 3 group being significantly higher than those in the S ≤ 2 group, while UA levels were significantly lower. The two groups showed no significant differences in other indicators at baseline (all $P > 0.05$).

Table 1. Comparison of Baseline Data in Chronic Hepatitis B Combined with Non-alcoholic Fatty Liver Disease Patients with Different Fibrosis Stages [a]

Descriptive Item	All Patients (n = 99)	S ≤ 2 (n = 63)	S ≥ 3 (n = 36)	t/Z/χ^2-Value	P-Value
Gender (No. %)				$\chi^2 = 0.054$	0.816
Male	87 (87.9)	55 (87.3)	32 (88.9)		
Female	12 (12.1)	8 (12.7)	4 (11.1)		
Inflammation grade (No. %)				$\chi^2 = 0.577$	0.447
G ≤ 2	98 (99.0)	62 (98.4)	36 (100.0)		
G ≥ 3	1 (1.0)	1 (1.6)	0 (0.0)		
Steatosis grade (No. %)				$\chi^2 = 3.158$	0.076
F < 2	69 (69.7)	40 (64.5)	29 (80.6)		
F ≥ 2	30 (30.3)	23 (36.5)	7 (19.4)		
Age (y; mean ± SD)	37.10 ± 8.26	35.59 ± 7.98	39.75 ± 8.19	$t = 0.046$	0.015
< 30	17 (17.2)	13 (20.6)	4 (11.1)	$\chi^2 = 1.461$	0.227
≥ 30	82 (82.8)	50 (79.4)	32 (88.9)		
ALT (U/L)	53.0 (39.0 - 89.0)	53.0 (41.0 - 89.0)	50.5 (35.3 - 91.5)	Z = -0.040	0.968
< 40	25 (25.3)	14 (22.2)	11 (30.6)	$\chi^2 = 0.843$	0.359
≥ 40	74 (74.7)	49 (77.8)	25 (69.4)		
HBeAg (S/CO)	4.75 (0.42 - 238.0)	4.75 (0.56 - 1072.0)	3.79 (0.39 - 157.9)	Z = -0.737	0.461
Positive	56 (60.9)	35 (61.4)	21 (60.0)	$\chi^2 = 0.018$	0.893
Negative	36 (39.1)	22 (38.6)	14 (40.0)		
BMI (kg/m^2)	25.62 ± 3.08	25.50 ± 2.61	25.83 ± 3.82	$t = 4.510$	0.681
< 23	9 (13.6)	5 (11.9)	4 (16.7)	$\chi^2 = 0.458$	0.795
23 ~ 25	18 (27.3)	11 (26.2)	7 (29.2)		
≥ 25	39 (59.1)	26 (61.9)	13 (54.2)		
WBC (10^9/L)	6.32 (5.64 - 7.64)	6.55 (5.65 - 8.24)	6.24 (5.16 - 7.35)	Z = -1.749	0.080
PLT (10^9/L)	187.0 (146.0 - 210.0)	189.0 (149.0 - 219.0)	180.5 (133.5 - 194.5)	Z = -1.702	0.089
MPV (fL)	10.7 (10.1 - 11.4)	10.7 (10.0 - 11.3)	10.7 (10.2 - 11.8)	Z = -0.522	0.602
AST (U/L)	33.0 (26.0 - 48.0)	32.0 (25.0 - 47.0)	37.0 (27.3 - 50.3)	Z = -1.077	0.281
GGT (U/L)	36.0 (24.0 - 58.0)	34.5 (24.0 - 54.3)	40.0 (24.3 - 97.0)	Z = -0.888	0.374
ALB (g/L)	44.6 (42.4 - 46.9)	44.6 (43.1 - 47.1)	44.3 (40.8 - 46.8)	Z = -1.285	0.199
ALP (U/L)	80.5 (65.0 - 101.8)	78.0 (64.5 - 99.0)	83.0 (65.0 - 113.0)	Z = -0.856	0.392
GLU (mmol/L)	5.07 (4.63 - 5.44)	5.13 (4.80 - 5.51)	4.89 (4.54 - 5.30)	Z = -1.627	0.104
CHOL (mmol/L)	4.67 (4.05 - 5.31)	4.82 (4.06 - 5.39)	4.55 (4.01 - 5.09)	Z = -1.225	0.221
TG (mmol/L)	1.35 (0.91 - 1.97)	1.46 (0.91 - 1.97)	1.30 (0.92 - 2.12)	Z = -0.302	0.763
TB (μmol/L)	16.30 (12.61 - 20.62)	16.76 (12.23 - 21.03)	15.85 (13.13 - 19.60)	Z = -0.129	0.898
DB (μmol/L)	4.6 (3.6 - 5.6)	4.6 (3.6 - 5.8)	4.6 (3.9 - 5.4)	Z = -0.579	0.563
UA (mmol/L)	369.5 (320.8 - 418.0)	377.5 (341.3 - 420.0)	335.0 (281.5 - 393.3)	Z = -2.045	0.041
AFP (ng/mL)	3.69 (2.26 - 5.64)	3.17 (2.07 - 5.16)	4.48 (2.97 - 8.33)	Z = -2.396	0.017
HA (ng/mL)	76.27 (51.68 - 125.86)	67.52 (43.78 - 116.09)	99.58 (58.00 - 139.97)	Z = -1.647	0.100
PIIIP (ng/mL)	21.37 (18.06 - 25.81)	21.28 (17.87 - 24.52)	22.38 (18.07 - 30.88)	Z = -1.189	0.234
CIV (ng/mL)	21.04 (18.15 - 26.06)	20.88 (18.03 - 23.53)	21.80 (18.31 - 28.73)	Z = -1.046	0.296
LN (ng/mL)	32.77 (24.02 - 39.78)	31.98 (21.10 - 37.67)	33.43 (27.42 - 51.01)	Z = -1.586	0.113
HBV DNA (log_{10}, IU/mL)	5.24 (3.01 - 7.63)	4.70 (2.89 - 7.75)	5.63 (3.64 - 7.29)	Z = -0.671	0.502
HBsAg (IU/mL)	3997.5 (1284.3 - 7350.3)	4186.0 (1132.0 - 10108.6)	3844.0 (1516.7 - 6831.5)	Z = -0.235	0.814
AAR	0.62 (0.50 - 0.82)	0.61 (0.50 - 0.80)	0.66 (0.47 - 0.89)	Z = -0.491	0.623
APRI	0.45 (0.33 - 0.85)	0.43 (0.33 - 0.65)	0.49 (0.40 - 0.92)	Z = -1.754	0.079
FIB-4	0.95 (0.65 - 1.43)	0.88 (0.60 - 1.20)	1.08 (0.80 - 1.94)	Z = -2.313	0.021
GPR	0.48 (0.27 - 0.75)	0.46 (0.25 - 0.68)	0.57 (0.31 - 1.40)	Z = -1.762	0.078
Forns Index	4.69 (3.61 - 5.77)	4.48 (3.30 - 5.47)	5.15 (3.84 - 6.08)	Z = -2.237	0.025
S-Index	0.12 (0.06 - 0.19)	0.10 (0.06 - 0.16)	0.13 (0.07 - 0.39)	Z = -1.956	0.050
NFS	0.33 (-0.26 - 0.91)	0.33 (-0.46 - 0.83)	0.47 (-0.13 - 1.72)	Z = -1.113	0.266

Abbreviations: CHB, chronic hepatitis B; NAFLD, non-alcoholic fatty liver disease; ALT, alanine aminotransferase; HBeAg, hepatitis B envelope antigen; BMI, Body Mass Index; WBC, white blood cell; PLT, platelet; MPV, mean platelet volume; AST, aspartate aminotransferase; GGT, glutamyl transpeptidase; ALB, albumin; ALP, alkaline phosphatase; GLU, glucose; CHOL, cholesterol; TG, triglyceride; TB, total bilirubin; DB, direct bilirubin; UA, uric acid; AFP, alpha fetoprotein; HA, hyaluronidase; PIIIP, procollagen III; CIV, type IV collagen; LN, laminin; HBV DNA, hepatitis B virus deoxyribonucleic acid; HBsAg, hepatitis B surface antigen; AAR, aspartate aminotransferase-to-alanine aminotransferase ratio; APRI, aspartate aminotransferase-to-platelet Ratio Index; FIB-4, Fibrosis Index based on the four factors; GPR, glutamyl transpeptidase-to- platelet ratio; NFS, NAFLD fibrosis score.

[a] Values are presented as median [interquartile ranges (IQR; P25 - P75)] unless otherwise indicated.

4.2. Correlation of Single-Factor Indicators with Fibrosis Stage and Their Diagnostic Efficacy

Spearman's rank correlation was used to analyze the relationship between fibrosis stage and indicators with $P < 0.15$ in the univariate analysis or previously established markers. According to the results (Table 2), fibrosis stage correlated significantly positively with age ($r = 0.257$, $P = 0.010$), AFP ($r = 0.295$, $P = 0.015$), FIB-4 ($r = 0.234$, $P = 0.020$), and Forns Index ($r = 0.228$, $P = 0.025$), and significantly negatively with UA ($r = -0.262$, $P = 0.040$). Additionally, the fibrosis stage correlated negatively with WBC, PLT, and GLU levels, and positively with HA, LN, APRI, and S Index, although the correlations were not significant (all $P > 0.05$). The performance of these unifactorial indexes in diagnosing fibrosis staging was further evaluated using ROC curves, revealing that the area under the curve (AUC) values of the indexes used to independently diagnose S ≥ 3 ranged between 0.600 and 0.670. At optimal cut-off values of 33.50 years, 7.63×10^9/L, 198.0×10^9/L, and 0.730 for age, WBC, PLT, and FIB-4, the sensitivities were found to be greater at 80.6%, 91.7%, 83.3%, and 83.3%, respectively. On the other

Table 2. Spearman's Rank Correlation and Receiver Operating Characteristic Analysis Between Single Factors and Fibrosis Stage

Descriptive Item	Spearman's Rank Correlation		ROC Analysis						
	r-Value	P-Value	AUC (95% CI)	P-Value	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Age	0.257	0.010	0.654 (0.542 - 0.767)	0.011	33.5	80.6	47.6	46.8	81.1
WBC	-0.177	0.080	0.606 (0.492 - 0.720)	0.080	7.63	91.7	34.9	44.6	88.0
PLT	-0.172	0.089	0.603 (0.489 - 0.717)	0.089	198.0	83.3	42.9	45.5	81.8
UA	-0.262	0.040	0.658 (0.498 - 0.818)	0.041	302.05	36.4	97.6	88.9	74.1
AFP	0.295	0.015	0.673 (0.539 - 0.806)	0.017	5.71	42.9	89.7	75.0	68.6
GLU	-0.175	0.104	0.604 (0.479 - 0.728)	0.104	4.795	44.1	77.4	55.6	68.3
HA	0.208	0.100	0.622 (0.480 - 0.764)	0.100	76.27	65.4	60.5	53.1	71.9
LN	0.200	0.113	0.617 (0.475 - 0.759)	0.113	40.82	38.5	89.5	71.4	68.0
AAR	0.050	0.626	0.530 (0.406 - 0.653)	0.623	0.675	50.0	63.5	43.9	69.0
APRI	0.177	0.079	0.606 (0.490 - 0.723)	0.080	0.395	77.8	44.4	44.4	77.8
FIB-4	0.234	0.020	0.640 (0.527 - 0.754)	0.021	0.730	83.3	41.3	44.8	81.3
GPR	0.179	0.228	0.607 (0.491 - 0.723)	0.078	0.855	36.1	83.9	56.5	69.3
Forns Index	0.228	0.025	0.636 (0.522 - 0.750)	0.025	4.755	63.9	61.3	48.9	74.5
S-Index	0.200	0.050	0.619 (0.502 - 0.736)	0.051	0.295	30.6	90.2	64.7	68.8
NFS	0.146	0.269	0.587 (0.434 - 0.740)	0.266	1.695	27.3	94.6	75.0	68.6

Abbreviations: ROC, receive operating characteristic; WBC, white blood cell; PLT, platelet; UA, uric acid; AFP, alpha fetoprotein; GLU, glucose; HA, hyaluronidase; LN, laminin; AAR, aspartate aminotransferase-to-alanine aminotransferase ratio; APRI, aspartate aminotransferase-to-platelet Ratio Index; FIB-4, Fibrosis Index based on the four factors; GPR, glutamyl transpeptidase-to- platelet ratio; NFS, NAFLD fibrosis score; AUC, area under curve; PPV, positive predictive value; NPV, negative predictive value.

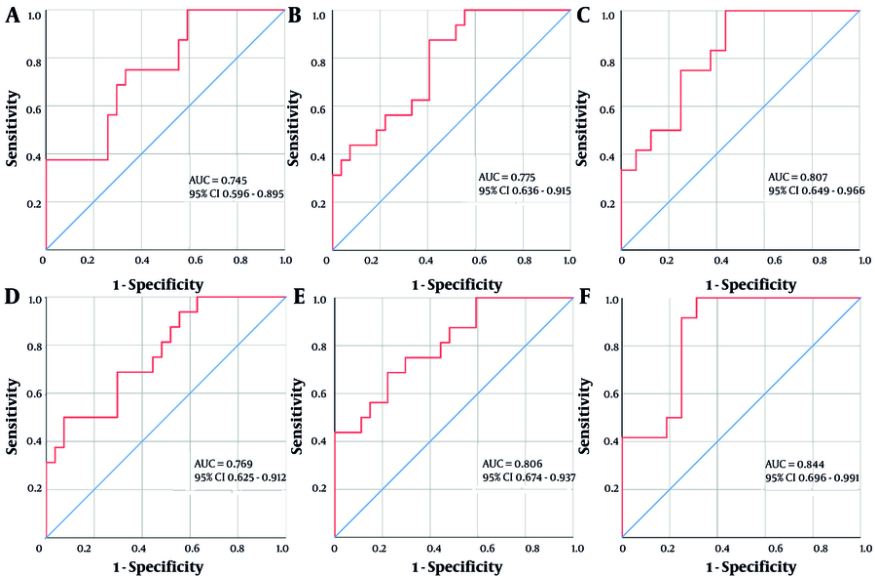

Figure 1. Receiver operating characteristic (ROC) curve analysis of multifactorial combinations predicting the risk of S ≥ 3 fibrosis in chronic Hepatitis B (CHB) combined with non-alcoholic fatty liver disease (NAFLD). A, AUA (age + UA + AFP); B, AUAWP (age + UA + AFP + WBC + PLT); C, AUAWPGHL (age + UA + AFP + WBC + PLT + GLU + HA + LN); D, AUAFF (age + UA + AFP + FIB-4 + Forns Index); E, AUAFFWPAGS (age + UA + AFP + FIB-4 + Forns Index + WBC + PLT + APRI + GPR + S-Index); and F, AUAFFWPAGSGHL (age + UA + AFP + FIB-4 + Forns Index + WBC + PLT + APRI + GPR + S-Index + GLU + HA + LN).

hand, at optimal thresholds, UA, AFP, LN, GPR, S-Index, and NFS exhibited high specificity values of 97.6%, 89.7%, 89.5%, 83.9%, 90.2%, and 94.6%, respectively. Furthermore, age, WBC, PLT, and FIB-4 had high NPV values (> 80.0%), with only UA showing a better PPV value (88.9%).

4.3. Correlation of Multifactorial Combined Indicators with Fibrosis Stage and Their Diagnostic Efficacy

Single-factor indicators with P-values less than 0.05, 0.10, and 0.15 in the ROC analyses were combined based on whether they contained previously established non-invasive diagnostic markers (APRI, FIB-4, GPR, Forns Index, and S-Index), and then designated as "AUA, AUAFF, AUAWP, AUAFFWPAGS, AUAWPGHL, and AUAFFWPAGSGHL", respectively. The Spearman's rank correlation analysis results suggested that these indicators were significantly positively correlated with

Table 3. Diagnostic Efficacy Analysis of Multiple Indicator Combinations and Fibrosis Stage [a]

Descriptive Items	Spearman's Rank Correlation		ROC Analysis								
	r-Value	P-Value	AUC (95% CI)	P-Value	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Z-Value [b]	P-Value [b]
P < 0.05										0.627	0.531
AUA	0.411	0.006	0.745 (0.596 - 0.895)	0.008	-0.484	68.8	70.4	57.9	79.2		
AUAFF	0.450	0.002	0.769 (0.625 - 0.912)	0.004	0.474	50.0	92.6	80.0	75.8		
P < 0.10										0.966	0.334
AUAWP	0.461	0.002	0.775 (0.636 - 0.915)	0.003	-0.993	87.5	59.3	56.0	88.9		
AUAFFWPAGS	0.512	0.000	0.806 (0.674 - 0.937)	0.001	-0.463	68.8	77.8	64.7	80.8		
P < 0.15										0.653	0.514
AUAWPGHL	0.527	0.004	0.807 (0.649 - 0.966)	0.006	-1.526	100.0	50.0	60.0	100.0		
AUAFFWPAGSGHL	0.590	0.001	0.844 (0.696 - 0.991)	0.002	-0.954	91.7	75.0	73.3	92.3		

Abbreviations: ROC, receive operating characteristic; AUC, area under curve; PPV, positive predictive value; NPV, negative predictive value.

[a] The regression equation established as follow: Logit (P_{AUA}) = -1.409 + 0.096*age-0.008*UA + 0.015*AFP, logit (P_{AUAFF}) = -2.071 + 0.081*age -0.007*UA + 0.010*AFP + 0.643*FIB -4 + 0.007*Forns Index, logit (P_{AUAWP}) = -1.205 + 0.109*age -0.006*UA + 0.009*AFP -0.204*WBC -0.002*PLT, logit ($P_{AUAFFWPAGS}$) = -5.148 + 0.028*age -0.007*UA + 0.006*AFP + 1.418*FIB -4 + 0.506*Forns Index -0.074*WBC + 0.017*PLT -1.011*APRI -2.228*GPR + 7.618*S-Index, logit ($P_{AUAWPGHL}$) = 1.473 + 0.060*age -0.005*UA + 0.007*AFP -0.021*WBC -0.001*PLT -0.722* GLU + 0.011*HA + 0.016*LN, logit ($P_{AUAFFWPAGSGHL}$) = -6.671 -0.049*age -0.004*UA + 0.000*AFP + 1.906*FIB -4+0.972*Forns Index + 0.220*WBC + 0.029*PLT -4.100*APRI -1.935*GPR + 14.898*S-Index -1.151*GLU + 0.026*HA + 0.021*LN.

[b] Comparison of diagnostic efficiency in specified groups of multiple indicator combinations.

the fibrosis stage ($0.4 < r < 0.6$, $P < 0.01$). Furthermore, ROC analysis of their diagnostic efficacy in patients with $S \geq 3$ CHB + NAFLD showed that the AUC values increased to a 0.740 ~ 0.850 range (Figure 1). At the optimal threshold, AUAFF and AUAWP demonstrated higher specificity (92.6%) and sensitivity (87.5%) values, respectively. Furthermore, the diagnostic sensitivities of AUAWPGHL and AUAFFWPAGSGHL were 100% and 91.7%, respectively, with PPVs ranging from 60.0% to 75.0% and NPVs being > 90.0%. Moreover, DeLong's test showed no significant difference in ROC curve performances for the multifactorial combined indicators across the different P-value classifications (all $P > 0.05$) (Table 3).

4.4. Correlation of Serological Markers with Steatosis Grade and Their Diagnostic Efficacy

Steatosis grading in CHB + NAFLD patients correlated positively with WBC, AST, ALB, and GLU, and negatively with MPV, LN, and HBeAg status, with only WBC, GLU, and LN exhibiting statistically significant correlation coefficients (all $P < 0.05$). The AUC values for these indicators' use in diagnosing $F \geq 2$ CHB + NAFLD ranged from 0.600 to 0.680, with high sensitivity (80.0%, 81.5%) and NPV (83.3%, 83.9%), especially when the critical values of ALB and GLU were 43.65 g/L and 4.81 mmol/L, respectively. Furthermore, LN had high specificity (83.7%) and NPV (81.8%) at an optimal cut-off value of 27.03 ng/mL. These indicators were further combined and designated as "WGL, WGLMA, and WGLMAAH" based on classifications of P-values less than 0.05, 0.10, and 0.15, respectively. The combinations correlated significantly positively with steatosis grading ($0.28 < r < 0.45$, all $P < 0.05$), and their AUC values for diagnosing $F \geq 2$ increased to the 0.670 ~ 0.800 range (Figure 2). Furthermore, they all had good specificity (> 902890.0%) and high PPV and NPV (> 75%). According to DeLong'sDeLong's test results, WGLMAAH showed a significantly better ROC curve performance than WGLMA ($Z = 2.073$, $P = 0.038$), and there were no significant differences between the two and WGL (all $P > 0.05$) (Table 4).

5. Discussion

Although the HBV infection-related mortality rate has declined globally, hepatocellular carcinoma (HCC)-related deaths have increased by 25%, with age-standardized death rates attributable to non-alcoholic steatohepatitis (a severe form of NAFLD) and alcohol abuse increasing the fastest (17). According to research, NAFLD accounts for approximately 30% of HCC cases in developed countries (18). Furthermore, chronic HBV infection superimposed on NAFLD has demonstrated a growing trend in Asia (2). Comorbid fatty liver has also been established as an independent risk factor for fibrosis in CHB patients (7). Moreover, fibrosis has been most closely associated with long-term adverse events (8). These insights imply the increasing significance of the degree of fibrosis and steatosis in the prediction of clinical outcomes in CHB + NAFLD patients. Therefore, developing non-invasive diagnostic methods for assessing fibrosis and steatosis in CHB + NAFLD patients is essential.

In this study, CHB + NAFLD patients were mostly male and had a mild inflammatory grading ($G \leq 2$). However,

some patients already presented with varying degrees of hepatic tissue pathology despite having normal ALT levels, implying that aminotransferase levels may not sufficiently predict pathologic changes in liver tissues, aligning with prior reports (19). Our findings also showed that fibrosis staging in CHB + NAFLD patients correlated positively with age and AFP, and negatively with UA levels. Furthermore, the steatosis grading was positively correlated with WBC and GLU, but negatively correlated with LN. These findings are consistent with previous research, which showed that age, gender, BMI, glucose metabolism disorders, dyslipidemia, and UA are independent predictors of NAFLD complications in CHB patients (20). Our findings also align with another study from mainland China, which concluded that hyperuricemia correlated negatively with significant hepatic fibrosis (S ≥ 2), and that UA was a protective factor for significant hepatic injury in CHB + NAFLD patients (21). However, the precise mechanisms remain unclear, potentially due to limited relevant research. Moreover, there is currently no conclusive evidence on how CHB and NAFLD affect each other in a combined state.

In this study, the steatosis grading of CHB + NAFLD patients correlated negatively with LN, a marker of liver fibrosis, implying that comorbid NAFLD may reduce the degree of fibrosis. These findings align with prior studies indicating that lower fibrosis stages correlate with a higher risk of steatosis, while comorbid NAFLD may protect against significant hepatic fibrosis (22, 23). This phenomenon could also be attributed to the mild degree of steatosis in our study cohort (> 2/3 were grade F1). Moreover, HBV infection is negatively associated with blood lipid profiles, and its metabolic modifications might prevent the progression of fatty liver (24). Evidence from recent studies has shown that persistent severe hepatic steatosis may be positively associated with fibrosis progression (6, 25). Overall, the interaction between CHB and NAFLD in a combined state remains largely unclear, necessitating significant additional research.

In this study, relevant unifactorial indicators and existing serological diagnostic models demonstrated low diagnostic value for detecting advanced fibrosis and moderate-to-severe steatosis in CHB + NAFLD patients, with AUCs ranging from 0.600 to 0.680. Our findings are consistent with previous studies in CHB + NAFLD patients, which demonstrated comparable diagnostic performance for advanced fibrosis: FIB-4 (AUC = 0.67), APRI (0.60), and NFS (0.65) (10). Compared to single serologic indicators, existing serologic models showed comparable accuracy in diagnosing advanced fibrosis, but significantly inferior effectiveness in diagnosing CHC, CHB, and NAFLD, possibly influenced by the dual etiologic background. According to recent research, chronic liver diseases of different etiologies have different pathogenesis and fibrosis patterns, necessitating different systems for fibrosis evaluation (26, 27). Although their diagnostic accuracy was not high, at the optimal threshold, some indicators such as age, WBC, PLT, and FIB-4 had sensitivity values > 80.0%. At the same time, the specificity values of UA, AFP, LN, S-Index, and NFS were near or above 90.0%. These findings suggest that the aforementioned indicators have a better role in identifying or excluding significant fibrosis.

We also found that the PPVs of existing serologic models for diagnosing significant fibrosis were generally low, with all PPVs being < 50.0%, except for those of GPR, S-Index, and NFS. Indeed, low PPV is a common problem with non-invasive liver fibrosis models and, as per the WHO-issued HBV guidelines, all non-invasive tests currently used to diagnose liver fibrosis and cirrhosis are considered to have a low PPV (< 50.0%) (11, 28). We further integrated different commonly used and serologic indicators in a multifactorial combination, yielding improved AUC and PPV values, with AUAWPGHL and AUAFFWPAGSGHL showing the highest sensitivity for diagnosing S ≥ 3 fibrosis. On the other hand, the specificity was reduced probably due to the involvement of multiple indicators susceptible to various factors. Furthermore, the multifactor combination indicators in different P-value categories showed no significant differences in ROC curve performances, implying that using combinations containing established non-invasive diagnostic markers may yield no significant advantage. Moreover, we found that the multifactorial combination used to diagnose moderate-to-severe steatosis had an AUC value of about 0.700 or more, as well as high specificity, PPV, and NPV, highlighting its usefulness in excluding obvious steatosis. A study evaluating noninvasive diagnostics for NASH with significant fibrosis reported AUC values of 0.739 (FibroScan-AST), 0.754 (LSM), 0.643 (NFS), and

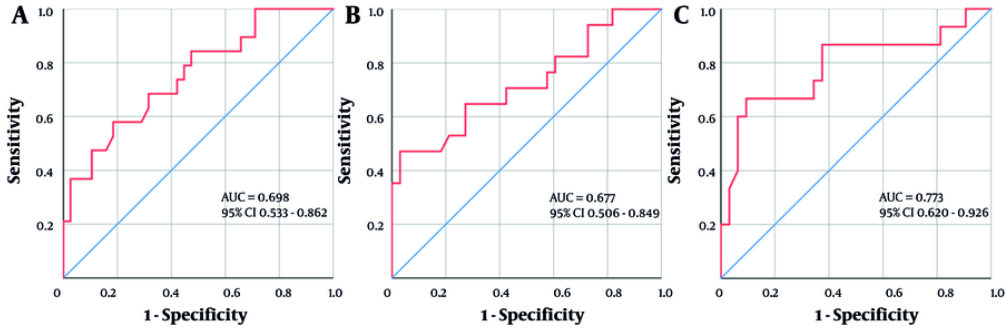

Figure 2. Receiver operating characteristic (ROC) curve analysis of multifactorial combinations predicting the risk of F ≥ 2 steatosis in chronic Hepatitis B (CHB) combined with non-alcoholic fatty liver disease (NAFLD). A, WGL (WBC + GLU + LN); B, WGLMA (WBC + GLU + LN + MPV + AST); and C, WGLMAAH (WBC + GLU + LN + MPV + AST + ALB + HBeAg status).

Table 4. Spearman's Rank Correlation and Receiver Operating Characteristic Analysis Between Single/Multiple Factors Combinations and Steatosis Grade

Descriptive Item	Spearman's Rank Correlation		ROC Analysis						
	r-Value	P-Value	AUC (95% CI)	P-Value	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
WBC	0.293	0.003	0.684 (0.566 - 0.802)	0.004	7.35	56.7	76.8	51.5	80.3
MPV	-0.181	0.092	0.614 (0.481 - 0.747)	0.092	10.45	53.8	67.7	41.2	77.8
AST	0.181	0.073	0.614 (0.493 - 0.735)	0.073	39.50	53.3	72.5	45.7	78.1
ALB	0.162	0.113	0.601 (0.486 - 0.717)	0.112	43.65	80.0	44.8	39.3	83.3
GLU	0.249	0.020	0.656 (0.534 - 0.777)	0.021	4.81	81.5	43.3	39.3	83.9
LN	-0.255	0.042	0.657 (0.504 - 0.809)	0.043	27.03	61.9	83.7	65.0	81.8
HBeAg status	-0.174	0.096	-	-	-	-	-	-	-
WGL	0.319	0.024	0.698 (0.533 - 0.862)	0.025	-0.32	56.3	79.4	56.3	79.4
WGLMA [a]	0.287	0.044	0.677 (0.506 - 0.849)	0.045	0.034	43.8	94.1	77.8	78.0
WGLMAAH [a]	0.441	0.001	0.773 (0.620 - 0.926)	0.002	-0.224	62.5	91.2	76.9	83.8

Abbreviations: ROC, receive operating characteristic; WBC, white blood cell; MPV, mean platelet volume; AST, aspartate aminotransferase; ALB, albumin; GLU, glucose; LN, laminin; PPV, positive predictive value; NPV, negative predictive value.

[a] Significant differences were seen in comparisons of ROC curves between WGLMA and WGLMAAH. The regression equation established as follow: Logit (P_{WGL}) = -5.664 + 0.480*WBC + 0.615*GLU -0.047*LN, logit (P_{WGLMA}) = -1.468 + 0.422*WBC + 0.568*GLU -0.042*LN -0.351*MPV + 0.000*AST, logit ($P_{WGLMAAH}$) = -3.461 + 0.462*WBC + 0.513* GLU-0.016*LN -0.327*MPV + 0.003*AST + 0.046*ALB -1.085*HBeAg (positive = 1, negative = 2).

0.665 (FIB-4) (29). However, few investigations have explored novel serological markers for fibrosis and steatosis assessment in CHB + NAFLD patients.

Overall, we developed predictive models for advanced fibrosis and moderate-to-severe steatosis in CHB + NAFLD patients using common clinical parameters. Although these models demonstrated significant superiority over previously established non-invasive diagnostic markers, they could not achieve both high sensitivity and specificity. This study has inherent limitations, including its retrospective, single-center nature and modest sample size, which may affect the statistical power and generalizability of the results. Further investigations are warranted to discover and validate optimized biomarkers for precise disease stratification in these patients. Nonetheless, this study crucially informs the establishment of non-invasive diagnostic indicators for liver fibrosis and steatosis, and the resulting models could be useful in diagnosing diseases in resource-limited areas.

Footnotes

Authors' Contribution: F. C. and H. Z. Z. conceived and designed the study, analyzed data, and wrote the manuscript. R. R. Z., M. X. X., and Q. H. L. collected and analyzed the clinical data.

Conflict of Interests Statement: The authors declare no conflict of interest.

Data Availability: Data sharing not applicable to this article as no datasets were generated or analyzed during the present study.

Ethical Approval: Ethics Committee of Third People's Hospital of Shenzhen has approved the study protocol on December 30, 2018 (approval No. 2018-014).

Funding/Support: This work was funded by grants from the Shenzhen Science and Technology Program (JCYJ20230807110708018), the National Natural Science Foundation of China (82220108013, U22A20275) and the Shenzhen Medical Research Funds (B2302007).

Informed Consent: Informed consent was obtained from all participants.

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