

Original Article

Combined alpha-fetoprotein, protein induced by vitamin K absence or antagonist-II, and aspartate aminotransferase to platelet ratio index for early hepatocellular carcinoma screening in hepatitis B cirrhosis

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Abstract: Objective: To evaluate the diagnostic performance of combined serum alpha-fetoprotein (AFP), protein induced by vitamin K absence or antagonist-II (PIVKA-II), and aspartate aminotransferase to platelet ratio index (APRI) for early hepatocellular carcinoma (HCC) screening in patients with hepatitis B virus (HBV)-related cirrhosis, and to develop a nomogram for risk prediction. Methods: This retrospective study included 200 patients with HBV-related cirrhosis (80 with early HCC and 120 with cirrhosis alone). Serum AFP, PIVKA-II, AST, and platelet count were measured. A combined diagnostic model was constructed using multivariate logistic regression, adjusting for BMI and smoking. Optimal cutoff values were determined using the maximum Youden index. Model performance was assessed using receiver operating characteristic (ROC) curves, calibration, and decision curve analysis (DCA). The incremental value of APRI was quantified using net reclassification improvement (NRI) and integrated discrimination improvement (IDI). Stratified 10-fold cross-validation assessed model stability. Results: The combined model (AFP + PIVKA-II + APRI + BMI + Smoking) achieved an AUC of 0.970 (95% CI: 0.946-0.989), with a sensitivity of 85.0% and specificity of 98.3%. The addition of APRI yielded no statistically significant incremental diagnostic value (NRI = -0.013, IDI = 0.020, P = 0.064). Cross-validation showed a mean AUC of 0.959 ± 0.052 . Calibration was good (Brier score = 0.061), and DCA confirmed clinical utility. Conclusion: Combined detection of AFP and PIVKA-II significantly improves early HCC screening accuracy in HBV-related cirrhosis, outperforming either marker alone. APRI provides no significant incremental value and may serve as a supplementary marker.

Keywords: Hepatocellular carcinoma, hepatitis B virus-related cirrhosis, alpha-fetoprotein, PIVKA-II, APRI, diagnostic performance, retrospective study

Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer worldwide, ranking fifth in incidence and second in mortality among malignant tumors [1]. China has a high prevalence of hepatitis B, with approximately 70 million chronic hepatitis B virus (HBV) carriers, and a significant proportion will progress to hepatitis B cirrhosis and eventually develop HCC [2, 3]. Patients with hepatitis B cirrhosis are at high risk of HCC, with an annual incidence of approximately 2%-5% [3]. Patients with early-stage HCC who receive radical treatment (such as surgical resection or local abla-

tion) can achieve a 5-year survival rate of over 70%; while patients with advanced HCC typically have a median survival of less than one year [4]. Therefore, early screening and diagnosis in high-risk populations with hepatitis B cirrhosis is of crucial clinical significance for improving patient prognosis.

Currently, the most commonly used methods for early HCC screening in clinical practice include serum alpha-fetoprotein (AFP) testing and abdominal ultrasound [5]. However, ultrasound examination is affected by operator experience, patient body type, and ascites, and has limited detection rates for early small

Combined AFP, PIVKA-II, APRI for HCC screening in HBV cirrhosis

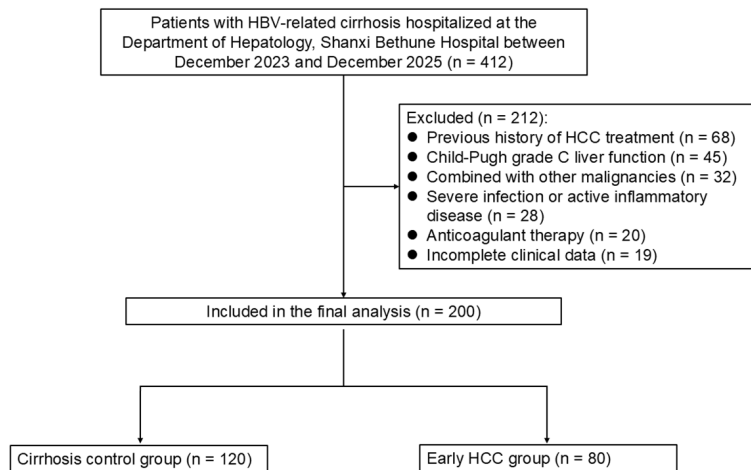


Figure 1. Flowchart of patient selection.

lesions. AFP, as a traditional serum marker for HCC, has a sensitivity of only 40%-60%, especially in early-stage HCC, where approximately 30%-40% of patients have AFP levels within the normal range [6]. Moreover, AFP may show non-specific elevation in the context of active hepatitis or liver cirrhosis, leading to increased false-positive rates [7]. Therefore, identifying novel or combined serum markers with higher diagnostic efficacy has become a key focus in the field of early HCC screening.

Protein induced by vitamin K absence or antagonist-II (PIVKA-II) is one of the most promising recently identified HCC serum markers. In HCC cells, decreased activity of vitamin K-dependent carboxylase prevents the normal conversion of prothrombin precursor into active prothrombin, resulting in its massive accumulation in serum [8]. Numerous studies have shown that PIVKA-II has higher sensitivity and specificity for HCC diagnosis than AFP, and provides important supplementary diagnostic value, especially in AFP-negative HCC patients [9, 10]. The aspartate aminotransferase to platelet ratio index (APRI) was initially used to assess liver fibrosis severity, but recent studies have found that APRI is closely related to HCC risk [11]. As a simple indicator calculated from routine biochemical parameters, APRI has the advantages of low cost, good repeatability, and ease of implementation in primary hospitals [12].

Although the value of AFP, PIVKA-II, and APRI in HCC diagnosis has been reported separately, systematic retrospective studies verifying the diagnostic value of combining these three markers for early HCC screening in patients

with hepatitis B cirrhosis are still lacking. Previous studies have mostly focused on single indicators or pairwise combinations, with small sample sizes or substantial population heterogeneity [13, 14]. Furthermore, the independent contribution of APRI in HCC screening and its complementary value with AFP and PIVKA-II have not been fully elucidated. Therefore, a retrospective study based on real-world clinical data is needed to systematically evaluate the clinical value of combined detection of AFP, PIVKA-II, and APRI

in early HCC screening among patients with hepatitis B cirrhosis.

Based on the above background, this study retrospectively collected clinical data from 200 patients with hepatitis B cirrhosis in order to explore the diagnostic efficacy of combined detection of serum AFP, PIVKA-II, and APRI in early HCC screening, and to construct a nomogram risk prediction model to provide a simple and economical screening tool for clinical decision-making.

Materials and methods

Research design and ethical review

This study is a retrospective case-control study. The research protocol was approved by the Ethics Committee of Shanxi Bethune Hospital. All procedures involving human participants were performed in accordance with the Declaration of Helsinki (as revised in 2013).

Research subjects

Patients with hepatitis B-related liver cirrhosis hospitalized in the Department of Hepatology at Shanxi Bethune Hospital from December 1, 2023 to December 31, 2025 were consecutively selected. A total of 412 patients were initially identified. According to pre-set inclusion and exclusion criteria, 200 patients were finally included for retrospective analysis. Based on clinical diagnosis results, they were divided into the early hepatocellular carcinoma (HCC) group (n = 80) and the simple liver cirrhosis control group (n = 120) (**Figure 1**).

Sample size calculation

Based on previous literature reports, the AUC for AFP in diagnosing HCC is approximately 0.75-0.80, and for PIVKA-II approximately 0.80-0.85. With α set to 0.05, β set to 0.20, an expected combined-model AUC of 0.88, and a case-to-control ratio of 1:1.5, we calculated the minimum required sample size as 70 HCC cases and 105 controls using PASS 15.0 software. This study actually included 80 cases of HCC and 120 controls, meeting the sample size requirements. Post-hoc power analysis based on the actual observed AUC of the combined model (0.970) and the final sample size (80 vs. 120) showed a statistical power exceeding 0.99, indicating that the study had sufficient statistical power to detect the observed differences.

Inclusion criteria: All patients (both the HCC group and the control group must meet the following requirements): (1) Age ≥ 18 years, gender not restricted; (2) Meet the diagnostic criteria for hepatitis B-related cirrhosis as stipulated in the Guidelines for the Prevention and Treatment of Chronic Hepatitis B (2022 Edition), confirmed by one of the following imaging methods: abdominal ultrasound, enhanced CT or MRI, or transient elastography (FibroScan) showing cirrhotic changes in the liver; (3) Hepatitis B surface antigen (HBsAg) positive for ≥ 6 months; (4) Complete clinical data, including fasting serum AFP, PIVKA-II, AST, alanine aminotransferase (ALT), platelet count (PLT), total bilirubin, albumin, prothrombin time, and other indicators measured on the morning of the day after admission; (5) Child-Pugh classification grade A or B (excluding patients with severe decompensation). For the early HCC group (case group), additional requirements: (6) Meet the clinical diagnostic criteria for early HCC as stipulated in the Diagnosis and Treatment Guidelines for Primary Liver Cancer (2022 Edition), i.e., BCLC stage 0 or A; (7) Confirmed as HCC by enhanced CT or MRI (arterial phase hyperenhancement, portal venous phase/delayed phase washout) or pathological biopsy (liver puncture or surgical specimen); (8) Maximum tumor diameter ≤ 5 cm, single or multiple but with a maximum of 3 nodules and maximum diameter ≤ 3 cm; (9) No tumor thrombus in the main portal vein or hepatic veins; (10) No extrahepatic metastasis.

For the simple cirrhosis control group, additional requirements: (11) Patients with hepatitis B-related cirrhosis hospitalized during the same period; (12) Confirmed without evidence of HCC by enhanced CT/MRI or abdominal ultrasound + AFP follow-up for ≥ 6 months; (13) No history of any HCC-related treatment.

Exclusion criteria: (1) Presence of other types of malignant tumors (such as cholangiocarcinoma, gallbladder cancer, metastatic liver cancer, pancreatic cancer, etc.); (2) Previous receipt of any HCC-related treatment (including but not limited to surgical resection, transhepatic arterial chemoembolization [TACE], radiofrequency ablation, microwave ablation, radiotherapy, targeted therapy, or immunotherapy); (3) Complicated with severe liver dysfunction (Child-Pugh grade C) or acute liver failure; (4) History of anticoagulation treatment (such as warfarin, rivaroxaban, etc., as it affects PIVKA-II levels); (5) Complicated with severe infection or active inflammatory diseases (such as sepsis, active tuberculosis, etc.); (6) Complicated with hematological diseases (such as leukemia, aplastic anemia, myelodysplastic syndrome, etc.) or severe coagulation dysfunction (INR > 2.0); (7) Complicated with human immunodeficiency virus (HIV) infection; (8) Missing key clinical indicators (AFP, PIVKA-II, AST, PLT, any one missing).

Data collection methods and procedures

Data were retrospectively extracted from the hospital's electronic medical record system and the laboratory information management system (LIS system). All data were independently extracted and entered into an Excel (Microsoft Office 2019) database by two researchers, then cross-checked after completion. In case of any inconsistencies, a third researcher arbitrated and resolved the issue. The data extraction period was from March 1, 2026 to April 30, 2026.

(1) General demographic data: Age: Calculated to the nearest year based on admission date; Gender: Male/Female; Body mass index (BMI) = Weight (kg)/Height² (m²); both height and weight were measured at admission; Smoking history: Defined as "smoking ≥ 1 cigarette/day for ≥ 1 year" or "cessation of smoking < 3 years"; Alcohol history: Defined as "daily alcohol consumption ≥ 40 g ethanol (for men) or $\geq$

20 g ethanol (for women), for ≥ 5 years” or “abstinence from alcohol < 3 years”; Diabetes history: Previously diagnosed or detected after admission with fasting blood glucose ≥ 7.0 mmol/L or glycated hemoglobin $\geq 6.5\%$; Hypertension history: Previously diagnosed or detected after admission with blood pressure $\geq 140/90$ mmHg.

(2) Laboratory indicators: All blood samples were collected in the early morning after admission (after 8-12 hours of fasting). The hospital laboratory department used chemiluminescent immunoassay to detect serum AFP (Roche Cobas e601, normal reference value < 7.0 ng/mL), chemiluminescent enzyme immunoassay to detect PIVKA-II (Abbott Architect i2000, normal reference value < 40 mAU/mL), enzyme rate method to detect AST and ALT (Roche Cobas c701, normal reference values < 40 U/L and < 50 U/L, respectively), automated hematology analyzer to detect PLT Sysmex XN-9000, normal reference value $125-350 \times 10^9/L$, vanadate oxidation method to detect total bilirubin (TBil) (Roche Cobas c701, normal reference value < 21 $\mu\text{mol/L}$), bromocresol green method to detect albumin (Roche Cobas c701, normal reference value 40-55 g/L), and clotting method to detect prothrombin time (PT) (Sysmex CS-5100, normal reference value 11-14.5 s). All test operations were carried out strictly in accordance with instrument operating procedures and reagent instructions, and were completed by professional laboratory personnel.

(3) Calculation of indicators: Based on laboratory test results, the APRI and albumin-bilirubin (ALBI) score were calculated. The formula for APRI is: $\text{APRI} = [(\text{AST}/\text{upper limit of normal (40 U/L)})/\text{PLT} (\times 10^9/L)] \times 100$. The formula for the ALBI score is: $\text{ALBI score} = -0.085 \times \text{albumin (g/L)} + 0.66 \times \log[\text{TBil} (\mu\text{mol/L})]$.

(4) Imaging and pathological data: For patients in the HCC group, tumor characteristics were retrospectively extracted from the imaging information system (PACS) and the pathological information system, including: maximum tumor diameter (measured on the maximum slice of enhanced CT/MRI arterial phase, in cm), number of tumors (single or multiple), presence of portal vein tumor thrombus (PVTT), BCLC stage (stage 0 or A), and liver cirrhosis morphology (nodular/atrophy, etc.). For patients in the control group, absence of tumor imaging evidence

was confirmed through the imaging information system. The same patient was required to undergo at least 2 imaging examinations with an interval of ≥ 3 months, and at least 1 of these examinations should be an enhanced CT or MRI.

Statistical analysis

All statistical analyses were conducted using SPSS 26.0 and R 4.2. A two-tailed $P < 0.05$ was considered statistically significant.

Continuous variables were expressed as mean $\pm$ standard deviation or median (interquartile range [IQR]), and compared using independent samples t-test or Mann-Whitney U test. Categorical variables were presented as frequencies (percentages) and compared using χ^2 test or Fisher's exact test. Continuous variables were compared between the two groups using the Mann-Whitney U test, as the data were not normally distributed (Shapiro-Wilk test, $P < 0.05$).

ROC curves were plotted for AFP, PIVKA-II, APRI, and the combined model, with AUC and 95% CI calculated via bootstrap resampling (1000 times). Optimal cutoff values were determined using the maximum Youden index. Multivariate logistic regression (stepwise forward) was used to construct the combined model, with AFP, PIVKA-II, APRI, BMI, and smoking status included. Model calibration was assessed using calibration curves, Brier score, and Hosmer-Lemeshow test. Stratified 10-fold cross-validation was performed to assess model stability. Net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were calculated to quantify the incremental value of APRI. Decision curve analysis (DCA) and clinical impact curves were used to assess clinical utility. A nomogram was plotted using the rms package in R.

Bias control measures

To control potential biases, the following measures were taken: (1) Selection bias: Patients were screened strictly according to pre-defined inclusion and exclusion criteria, and all screening processes were independently completed by two researchers; (2) Information bias: Laboratory tests were conducted by professional laboratory personnel under blind condi-

tions, and researchers were unaware of grouping results when collecting data; (3) Recall bias: This was a retrospective study, and all data originated from medical records, so there was no recall bias; (4) Confounding bias: In the comparison of baseline characteristics, potential confounding variables such as age, gender, and BMI were compared between groups, and multiple variables were included in logistic regression for multivariate adjustment; (5) Data entry bias: Data were independently entered by two researchers and cross-checked, with any inconsistencies arbitrated by a third researcher.

Results

Baseline characteristics

The age of the HCC group was slightly higher than that of the cirrhosis group [54.00 (46.75-57.25) years vs. 55.00 (51.00-60.00) years], but the difference was not statistically significant ($P = 0.058$). The BMI of the HCC group was significantly higher than that of the cirrhosis group [24.75 (22.59-27.18) kg/m² vs. 22.41 (20.49-24.53) kg/m², $P < 0.001$]. In terms of laboratory indicators, the levels of AFP, PIVKA-II, AST, total bilirubin, prothrombin time, APRI, and ALBI scores were significantly higher in the HCC group than in the cirrhosis group (all $P < 0.05$), while ALT showed no significant difference between the two groups ($P = 0.144$). In contrast, PLT and albumin levels were significantly lower in the HCC group compared with the cirrhosis group (all $P < 0.05$). Among categorical variables, the proportion of smokers was markedly higher in the HCC group compared with the cirrhosis group (58.8% vs. 30.0%, $P < 0.001$); there were no significant differences in gender, drinking history, diabetes, hypertension, or Child-Pugh classification distribution between the two groups (all $P > 0.05$) (**Table 1**).

Serum markers

The violin plot (**Figure 2**) visually presents the distribution differences of the three serum markers between the two groups. Specifically, **Figure 2A** shows that AFP levels in the HCC group [22.05 (16.38-26.32) ng/mL] were significantly higher than in the cirrhosis group [8.94 (4.11-13.67) ng/mL, $U = 1083.5$, $P < 0.001$]; **Figure 2B** shows that PIVKA-II levels in

the HCC group [59.42 (46.88-67.41) mAU/mL] were also significantly higher than in the cirrhosis group [29.63 (20.45-41.16) mAU/mL, $U = 936.5$, $P < 0.001$]; **Figure 2C** shows that APRI levels in the HCC group [121.66 (85.29-162.78)] were significantly higher than in the cirrhosis group [90.40 (64.85-130.14), $U = 3400.5$, $P < 0.001$]. Overall, all three markers exhibited significant distribution differences between the two groups.

Diagnostic efficacy of single indicators

The ROC curves for AFP, PIVKA-II, and APRI are shown in **Figure 3**, with detailed diagnostic metrics presented in **Table 2**. The AUC of AFP was 0.887 (95% CI: 0.844-0.926), with an optimal cutoff value of 13.88 ng/mL, corresponding to a sensitivity of 86.3%, specificity of 76.7%, and Youden index of 0.629. The AUC of PIVKA-II was 0.903 (95% CI: 0.857-0.941), with the optimal cutoff value being 42.24 mAU/mL, a sensitivity of 87.5%, specificity of 77.5%, and Youden index of 0.650. The AUC of APRI was 0.648 (95% CI: 0.567-0.722), with the optimal cutoff value being 101.96, a sensitivity of 70.0%, specificity of 60.8%, and Youden index of 0.308 (**Table 2**).

Diagnostic efficacy and calibration of the combined model

AFP, PIVKA-II, and APRI were included in the multivariate Logistic regression model, with BMI and smoking history adjusted, to construct a combined diagnostic model. The ROC curve (**Figure 3**) showed that the AUC of the combined model (adjusted) reached 0.970 (95% CI: 0.946-0.989), significantly higher than any single indicator (DeLong test, all $P < 0.001$). At the optimal cutoff value determined by the maximum Youden index (0.5958), the combined model achieved a sensitivity of 85.0%, specificity of 98.3%, positive predictive value of 97.1%, negative predictive value of 90.8%, and accuracy of 90.8% (**Table 2**). In 10-fold cross-validation, the combined model maintained robust performance with a mean AUC of 0.959 ± 0.052 . The full model (AFP + PIVKA-II + APRI + BMI + Smoking) achieved an AUC of 0.970; however, removing APRI did not significantly reduce performance (NRI = -0.013, IDI = 0.020, $P = 0.064$), suggesting that APRI provides limited incremental value.

Table 1. Baseline characteristics of the study population

Variable	Cirrhosis (n = 120)	HCC (n = 80)	U/ χ^2	P value
Age (years)	54.00 (46.75-57.25)	55.00 (51.00-60.00)	4040.5	0.058
BMI (kg/m ²)	22.41 (20.49-24.53)	24.75 (22.59-27.18)	2828.5	< 0.001
AFP (ng/mL)	8.94 (4.11-13.67)	22.05 (16.38-26.32)	1083.5	< 0.001
PIVKA-II (mAU/mL)	29.63 (20.45-41.16)	59.42 (46.88-67.41)	936.5	< 0.001
AST (U/L)	49.73 (37.38-64.27)	56.16 (39.58-77.21)	3933.0	0.031
ALT (U/L)	41.59 (32.14-53.51)	47.06 (33.78-57.17)	4214.0	0.144
Platelet ($\times 10^9$ /L)	134.62 (111.63-160.05)	119.38 (96.20-139.11)	6034.0	0.002
Total bilirubin (μ mol/L)	18.68 (11.80-24.67)	22.08 (14.23-30.34)	3829.0	0.016
Albumin (g/L)	39.16 (35.52-42.50)	35.83 (32.31-39.42)	6597.5	< 0.001
PT (s)	13.70 (12.61-14.63)	14.64 (13.14-15.86)	3529.0	0.002
APRI	90.40 (64.85-130.14)	121.66 (85.29-162.78)	3400.5	< 0.001
ALBI score	-1.50 (-1.99 to -1.10)	-1.07 (-1.57 to -0.68)	3099.0	< 0.001
Sex, n (%)				
Female	34 (28.3)	19 (23.8)	0.309	0.578
Male	86 (71.7)	61 (76.2)		
Smoking, n (%)				
No	84 (70.0)	33 (41.2)	15.180	< 0.001
Yes	36 (30.0)	47 (58.8)		
Alcohol, n (%)				
No	62 (51.7)	41 (51.2)	0.003	0.954
Yes	58 (48.3)	39 (48.8)		
Diabetes, n (%)				
No	104 (86.7)	61 (76.2)	2.922	0.087
Yes	16 (13.3)	19 (23.8)		
Hypertension, n (%)				
No	95 (79.2)	62 (77.5)	0.011	0.916
Yes	25 (20.8)	18 (22.5)		
Child-Pugh grade, n (%)				
A	79 (65.8)	45 (56.2)	1.486	0.223
B	35 (29.2)	27 (33.8)	0.281	0.596
C	6 (5.0)	8 (10.0)	1.155	0.282

Notes: Data are presented as median (Q1-Q3) for continuous variables and as number (percentage) for categorical variables. Comparisons between groups were performed using the Mann-Whitney U test for continuous variables, and the chi-square test or Fisher's exact test for categorical variables. *P*-values < 0.05 were considered statistically significant. Abbreviations: HCC, hepatocellular carcinoma; BMI, body mass index; AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist-II; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT, prothrombin time; APRI, AST to platelet ratio index; ALBI, albumin-bilirubin.

Compared with the dual-marker model (AFP + PIVKA-II), the addition of APRI yielded an NRI of -0.0125 and an IDI of 0.0196 (*P* = 0.064), suggesting that APRI provides marginal but non-significant incremental diagnostic value in the combined model.

The calibration curve (**Figure 4**) shows that the predicted probabilities of the combined model are in good agreement with actual observations. The Brier score was 0.061, the calibration

slope was 0.920, the calibration intercept was 0.040, and the χ^2 value of the Hosmer-Lemeshow test was 4.771 with *P* = 0.782 (**Table 3**).

Clinical decision value analysis

Decision curve analysis (**Figure 5; Table 4**) shows that within the threshold probability range of 10% to 30%, the net benefit of the combined model (adjusted) was higher than

Combined AFP, PIVKA-II, APRI for HCC screening in HBV cirrhosis

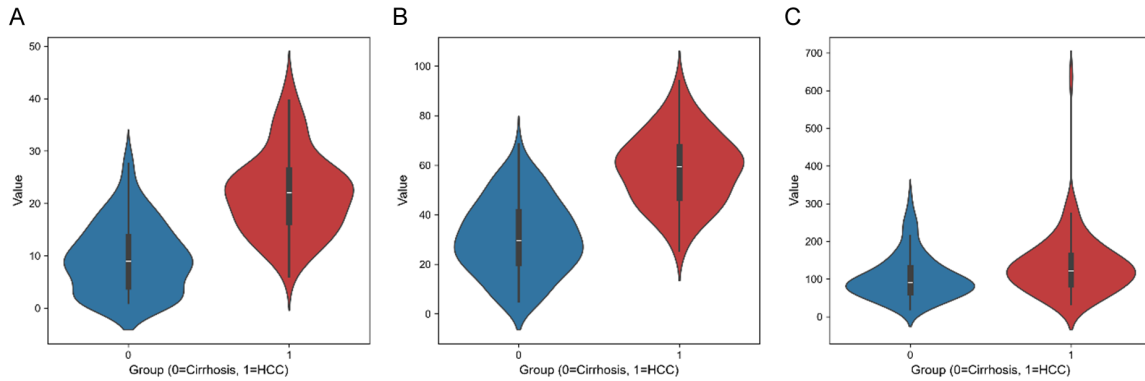


Figure 2. Violin plots showing the distribution differences of AFP, PIVKA-II, and APRI between cirrhosis and HCC groups. A. AFP (ng/mL); B. PIVKA-II (mAU/mL); C. APRI. The violin plots display the density distribution of each marker across the two groups. The box plots inside the violins indicate the median (horizontal line) and interquartile range (box). Group 0 = cirrhosis (n = 120); Group 1 = HCC (n = 80). All three markers showed statistically significant differences between the two groups ($P < 0.05$, Mann-Whitney U test). Abbreviations: AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist-II; APRI, AST to platelet ratio index; HCC, hepatocellular carcinoma.

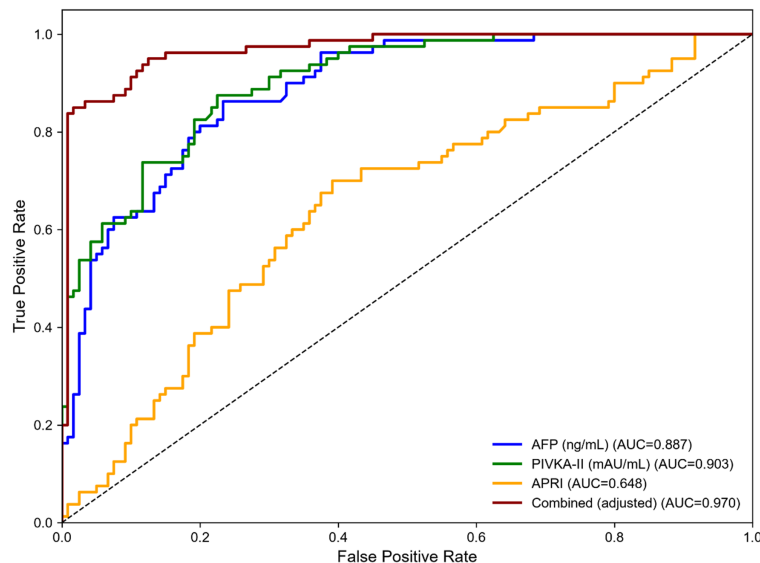


Figure 3. ROC curves of individual markers and the combined model. Abbreviations: AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist-II; APRI, AST to platelet ratio index; AUC, area under the curve.

that of AFP alone, PIVKA-II alone, and the “treat all” strategy. For example, at a threshold probability of 20%, the net benefit of the combined model was 0.357, while that of AFP was 0.320, PIVKA-II was 0.298, and APRI was 0.116. This indicates that the combined model has higher practical value in clinical decision-making.

The clinical impact curve (Figure 6) further illustrates the proportion of true positive cases

identified by the combined model at a given threshold probability. When the threshold probability was set at 10%, the model could identify 97.5% of HCC patients; when the threshold probability was raised to 30%, it could still identify 92.5% of HCC patients. This result further supports the application of the combined model in early HCC screening.

Construction of the nomogram prediction model

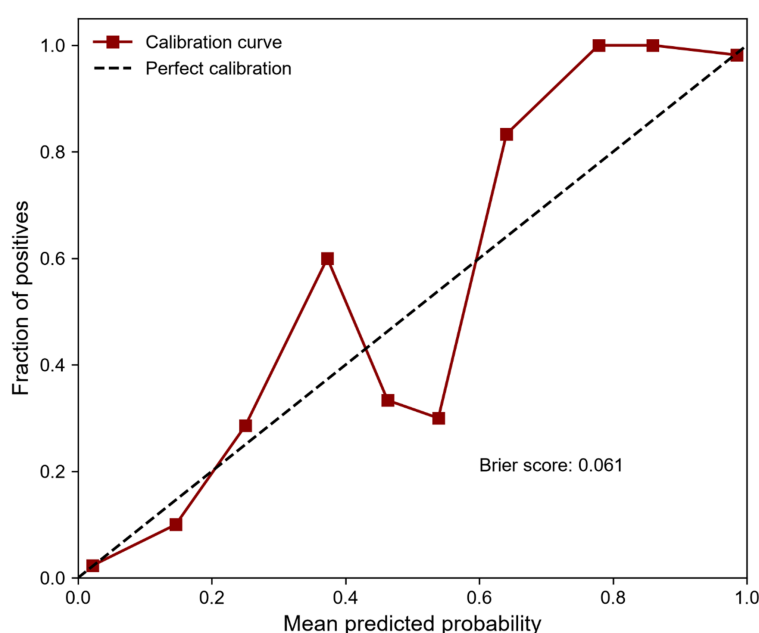
Based on the results of the multivariate Logistic regression model, we constructed an early HCC risk prediction nomogram (Figure 7). The variables included in the model are AFP, PIVKA-II, and

APRI, with regression coefficients of 0.242, 0.117, and 0.010, respectively, and the constant term is -15.059. Clinicians can read the corresponding individual score from the nomogram based on the specific values of these three indicators, sum them up, and obtain the predicted probability of HCC for individual patients through the total score axis mapping. This provides an intuitive and quantitative reference tool for clinical decision-making.

Table 2. Comparison of diagnostic performance of individual markers and the combined model (AFP + PIVKA-II + APRI + BMI + Smoking)

Marker	AUC (95% CI)	Cutoff	Sensitivity	Specificity	Youden index	PPV	NPV	Accuracy
AFP (ng/mL)	0.887 (0.844-0.926)	13.88	0.863	0.767	0.629	0.711	0.893	0.805
PIVKA-II (mAU/mL)	0.903 (0.857-0.941)	42.24	0.875	0.775	0.650	0.722	0.903	0.815
APRI	0.648 (0.567-0.722)	101.96	0.700	0.608	0.308	0.544	0.753	0.645
Combined model (adjusted)	0.970 (0.946-0.989)	0.60	0.850	0.983	0.833	0.971	0.908	0.908

The combined model included AFP, PIVKA-II, APRI, BMI, and smoking status. Notes: Optimal cutoff values were determined using the maximum Youden index. The area under the curve (AUC) and 95% confidence intervals (CI) were calculated using the bootstrap method with 1000 resamples. Abbreviations: AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist-II; APRI, AST to platelet ratio index; AUC, area under the curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

**Figure 4.** Calibration curve of the combined model.**Table 3.** Calibration metrics of the combined model

Metric	Value
Brier score	0.061
Calibration slope	0.920
Calibration intercept	0.040
Hosmer-Lemeshow chi-square	4.771
Hosmer-Lemeshow P value	0.782

Notes: The Brier score measures the mean squared difference between predicted probabilities and observed outcomes (lower values indicate better calibration). Calibration slope and intercept were derived from the calibration curve. The Hosmer-Lemeshow test was used to assess the goodness-of-fit; a *P*-value > 0.05 indicates adequate calibration.

Correlation analysis among markers

The correlation coefficient heatmap (**Figure 8**) shows the correlations between AFP, PIVKA-II, APRI, ALBI, PLT and the grouping variables. The correlation coefficient between AFP and the grouping variable was 0.632, and between PIVKA-II and the grouping variable was 0.684, indicating a strong positive correlation between both markers and HCC status. The correlation coefficient between AFP and PIVKA-II was 0.433, suggesting that the information of the two has some overlap but is not completely redundant. APRI had a strong negative correlation with PLT ($r = -0.567$), and a lower correlation coefficient with the grouping variable ($r = 0.177$).

Discussion

This study systematically evaluated the diagnostic value of AFP, PIVKA-II, and APRI for early HCC screening in HBV-related cirrhosis. Our findings confirm that the combination of AFP and PIVKA-II provides excellent diagnostic performance.

The advantages of PIVKA-II in HCC diagnosis have been confirmed in multiple studies [15-17]. PIVKA-II is an abnormal prothrombin that accumulates in serum due to decreased vita-

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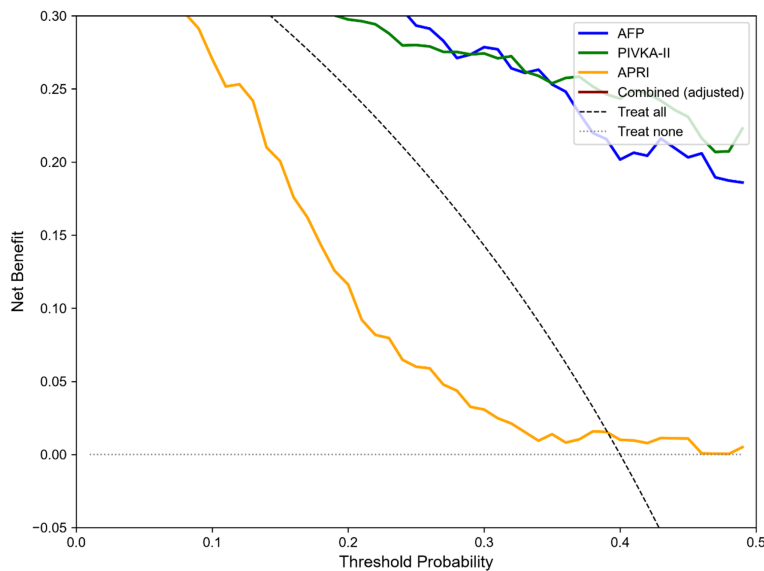


Figure 5. Decision curve analysis (DCA) comparing the net benefit of different models. Abbreviations: AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist-II; APRI, AST to platelet ratio index; DCA, decision curve analysis.

Table 4. Net benefit at different threshold probabilities from decision curve analysis

Model	Threshold	Net benefit
AFP	0.10	0.353
AFP	0.20	0.320
AFP	0.30	0.279
PIVKA-II	0.10	0.342
PIVKA-II	0.20	0.298
PIVKA-II	0.30	0.274
APRI	0.10	0.271
APRI	0.20	0.116
APRI	0.30	0.031
Combined model	0.10	0.370
Combined model	0.20	0.357
Combined model	0.30	0.342

Notes: Net benefit is calculated as the difference between the proportion of true positives and the proportion of false positives weighted by the threshold probability. Higher net benefit indicates greater clinical utility compared to the “treat all” or “treat none” strategies. Abbreviations: AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist-II; APRI, AST to platelet ratio index; DCA, decision curve analysis.

min K-dependent carboxylase activity in HCC cells, which prevents normal conversion of the prothrombin precursor [18, 19]. This pathological mechanism is closely related to the degree of tumor cell dedifferentiation, so the level of

PIVKA-II reflects tumor malignancy and proliferative activity to a certain extent. The diagnostic performance of PIVKA-II in our study was generally consistent with previous meta-analyses [20], although its specificity was lower than that reported in studies using healthy controls [21]. This discrepancy likely reflects the higher baseline PIVKA-II levels in cirrhotic patients due to impaired vitamin K-dependent carboxylation [22].

AFP remains a widely used screening marker; however, its limitations are also prominent. Firstly, the sensitivity of AFP in early HCC is only 40%-60%, and approximately 30%-40% of HCC patients have normal AFP levels [23]. Secondly,

in the context of active hepatitis and liver cirrhosis, AFP can show non-specific elevation, leading to an increase in false positive rates [24]. These limitations highlight the necessity of a multi-marker approach.

APRI was initially used to assess liver fibrosis severity, but recent studies have found that APRI is closely related to HCC risk [25, 26]. An increase in APRI reflects liver fibrosis progression and declining liver reserve function, and liver fibrosis itself is an independent risk factor for HCC [27]. Although APRI demonstrated limited diagnostic efficacy as a standalone marker, consistent with previous studies [28], it showed a weak but significant association with HCC risk in the multivariate model ($P = 0.016$), suggesting a potential supplementary role. Given its strong negative correlation with platelet count, a marker of portal hypertension and an independent HCC risk factor, APRI may indirectly signal HCC risk through platelet consumption. From a clinical application perspective, APRI is easy to calculate, has low cost, and does not require special equipment, making it suitable for widespread use in primary care settings as an auxiliary indicator.

The combination of AFP and PIVKA-II demonstrated superior diagnostic performance to any

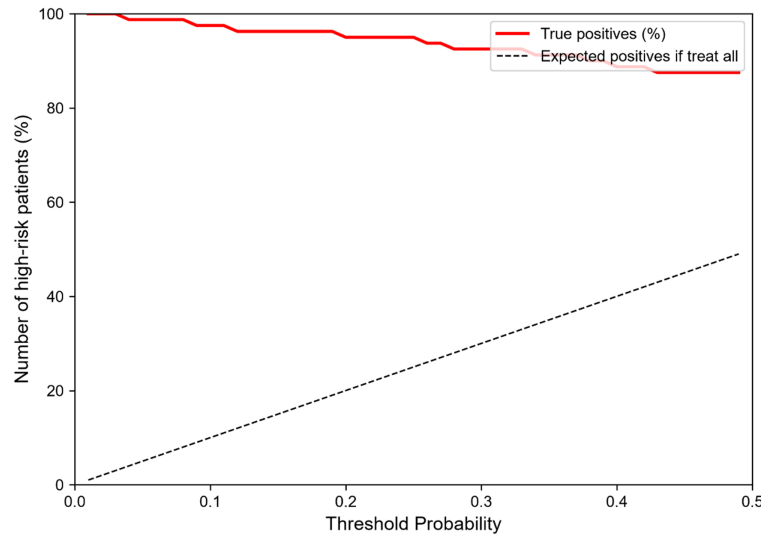


Figure 6. Clinical impact curve of the combined model.

single marker, with a balanced sensitivity and specificity profile suitable for screening purposes.

Compared with previous studies, the combined model in this study has certain advantages in diagnostic efficacy. A meta-analysis including 1328 HCC patients showed that the combined AUC of AFP and PIVKA-II was 0.93, slightly lower than 0.970 in this study [29]. Another prospective study on patients with hepatitis B-related liver cirrhosis reported that the sensitivity of AFP combined with PIVKA-II was 88.2% and the specificity was 92.5% [20, 30], which differed from the sensitivity (85.0%) and specificity (98.3%) of this study. These differences might be attributed to the baseline characteristics of the study population and the heterogeneity of the detection methods.

The constructed nomogram facilitates individualized risk assessment. For patients with high scores, more intensive surveillance (e.g., ultrasound every 3 months) is recommended, while patients with low scores can maintain routine screening intervals.

In this study, AST levels were significantly higher in the HCC group than in the cirrhosis group, whereas ALT levels did not differ significantly. This discrepancy may reflect mitochondrial damage and altered tumor metabolism in HCC, as the AST/ALT ratio is frequently elevated in patients with HCC and is associated with poorer prognosis.

This study has the following limitations. First, although we took measures such as double independent data extraction, cross verification, and multivariate adjustment to control bias, we could not completely eliminate the influence of selection and information bias due to the retrospective study design. Second, the single-center sample limits the generalizability of the study results. As a regional medical center, the patient population of Shanxi Bethune Hospital may have regional and demographic bias. Third, the cost of PIVKA-II detection is relatively high, which may

limit its promotion in primary hospitals under the current context of medical insurance cost control. Fourth, this study only included early HCC (BCLC 0/A stage). Although this helps evaluate screening value, it fails to reflect the application effect of the combined model in advanced HCC. Fifth, the follow-up time is limited, and the long-term predictive value of the combined model for HCC incidence cannot be evaluated, nor can the impact of dynamic changes of markers on prognosis be observed. In addition, this study did not include novel biomarkers such as circulating tumor DNA and microRNAs, and did not explore better multi-omics combined strategies.

Although the cost of PIVKA-II detection is higher than that of AFP, the specificity of the combined model could substantially reduce unnecessary imaging examinations and follow-ups. From a long-term perspective, the model may prove to be cost-effective by minimizing false-positive referrals and optimizing resource allocation.

Based on the results of this study, further exploration is recommended in the following directions. First, prospective multi-center cohort studies should be conducted to verify the stability and universality of the combined model across different ethnic groups and in populations with different liver disease backgrounds. Second, dynamic monitoring strategies should be explored to evaluate the value of serial measurements of AFP and PIVKA-II for the early

Combined AFP, PIVKA-II, APRI for HCC screening in HBV cirrhosis

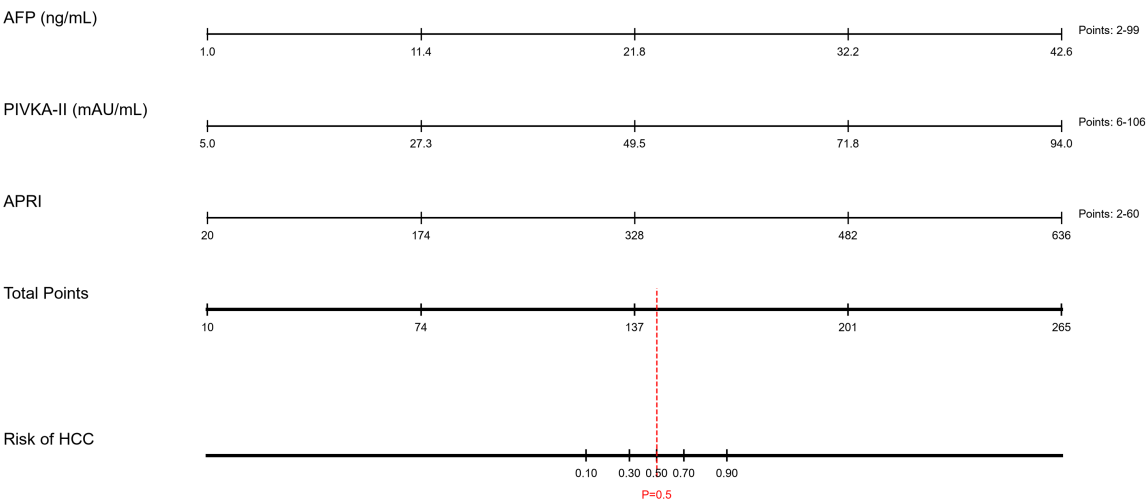


Figure 7. Nomogram for early HCC risk prediction based on AFP, PIVKA-II, and APRI. Abbreviations: AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist-II; APRI, AST to platelet ratio index; HCC, hepatocellular carcinoma.

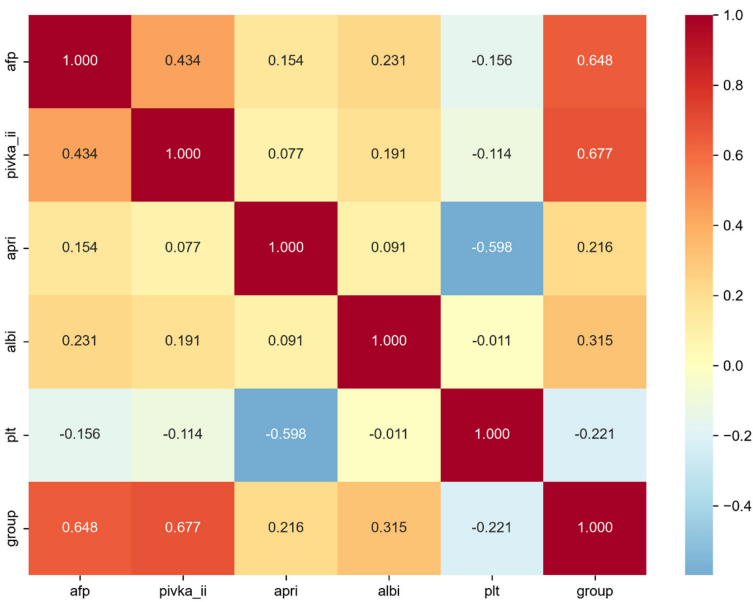


Figure 8. Correlation heatmap of serum markers and group variable. Abbreviations: AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist-II; APRI, AST to platelet ratio index; ALBI, albumin-bilirubin; PLT, platelet.

warning of HCC. Third, imaging biomarkers and artificial intelligence techniques should be integrated to construct a comprehensive diagnostic model incorporating serological indicators, imaging features, and clinical parameters. Fourth, cost-effectiveness analysis should be performed to assess the economic feasibility of

the combined model under current medical resource conditions.

Conclusion

The full model (AFP + PIVKA-II + APRI + BMI + Smoking) achieved an AUC of 0.970, with a sensitivity of 85.0% and specificity of 98.3%, significantly outperforming any single marker. The calibration is good, and clinical decision curve analysis confirms its high clinical practicability. The nomogram constructed based on the three indicators provides an intuitive individualized risk prediction tool, which helps clinicians optimize screening strategies and resource allocation. This combined model, as an effective tool for early screening of high-risk individuals with hepatitis B cirrhosis, is worthy of further verification and promotion in multi-center prospective cohorts.

Disclosure of conflict of interest

None.

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