Yan Lei, Xiaolan Lu, Xiuping Duan, Wei Tang* and Qiang Wang*

Bilirubin is a superior biomarker for hepatocellular carcinoma diagnosis and for differential diagnosis of benign liver disease

https://doi.org/10.1515/labmed-2023-0023
Received February 22, 2023; accepted August 16, 2023;
published online September 7, 2023

Abstract

Objectives: To develop a novel diagnostic model combining bilirubin, protein induced by vitamin K absence or antagonist-II (PIVKA-II), and alpha-fetoprotein (AFP) to improve hepatocellular carcinoma (HCC) diagnosis.
Methods: The serum levels of PIVKA-II, AFP, and bilirubin in 718 HCC patients and 2,763 benign liver disease (BLD) patients were measured. A mathematical model was used as the combined diagnostic model (PIVKA-II, AFP, and bilirubin: PAB combination) for improving HCC diagnosis. Receiver operating characteristic (ROC) curves were used to analyse the diagnostic value of the individual markers, the PIVKA-II and AFP (PA) combination, and the PAB combination for HCC diagnosis.
Results: With the increase in bilirubin, the positive predictive value (PPV) of bilirubin in HCC diagnosis decreased ($p<0.001$) while the negative predictive value (NPV) increased ($p<0.001$). The areas under the ROC curves (AUCs) of the PAB combination were 0.935 and 0.862 for the diagnosis of HCC and HCC<3.0 cm, respectively, which were significantly higher than those of PIVKA-II, AFP, and the PA combination ($p<0.001$). The AUC values for PIVKA-II, AFP, and the PA combination were significantly decreased for the diagnosis of HCC and HCC<3.0 cm when serum levels of PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL were used for diagnosis, while the AUC value of the PAB combination increased.
Conclusions: Bilirubin is a superior biomarker for diagnosing HCC and distinguishing HCC from BLD. The combination of bilirubin with PIVKA-II and AFP has superior diagnostic value for HCC and early-stage HCC.

Keywords: bilirubin; alpha-fetoprotein; protein induced by vitamin K absence or antagonist-II; benign liver disease; hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is the third-most common cause of cancer-related death, and the number of deaths due to HCC is approximately 700,000 every year globally. In China, HCC is the fourth-most common cancer and the third-most common cause of cancer death [1, 2]. Of new HCC cases worldwide, 50 % occur in China annually [3]. Chronic hepatitis B (CHB) is associated with an increased risk of HCC, and more than 50 % of global HCC cases and 60–80 % of HCC cases in Asia and Africa are related to CHB infection [4, 5].

The 5-year survival rate of patients with HCC is closely related to the stage at which HCC is diagnosed, with the rate exceeding 70 % for early-stage HCC and a rate lower than 10 % for advanced-stage HCC, and the median survival time is only 1–2 years [6–8]. Because of the difficulties in diagnosing early-stage HCC, most cases are diagnosed at an advanced stage, which is the main reason for the poor prognosis of patients with HCC [9, 10].

Alpha-fetoprotein (AFP) and protein induced by a vitamin K absence or antagonist-II (PIVKA-II) are widely used in HCC diagnosis [11–13]. These markers have been included in the guidelines for HCC diagnosis issued by many national hepatology societies [14–16]. However, in clinical application, approximately 50 % of HCC cases and 60–80 % of early-stage HCC cases do not express AFP, which is the main reason for the poor sensitivity of AFP in diagnosing HCC

Yan Lei and Xiaolan Lu contributed equally to this work.

***Corresponding authors: Wei Tang**, Sichuan Key Laboratory of Medical Imaging, Department of Radiology, Affiliated Hospital of North Sichuan Medical College, Nanchong 637000, Sichuan, P.R. China, E-mail: tw-n-g-up@163.com; and **Qiang Wang**, Department of Clinical Laboratory, Affiliated Hospital of North Sichuan Medical College, Nanchong, 637000, Sichuan, P.R. China; Department of Laboratory Medicine, North Sichuan Medical College, Nanchong, 637000, Sichuan, P.R. China; and Translational Medicine Research Center, North Sichuan Medical College, Nanchong, 637000, Sichuan, P.R. China, E-mail: wqiang_1981@126.com
Yan Lei and Xiaolan Lu, Department of Clinical Laboratory, Affiliated Hospital of North Sichuan Medical College, Nanchong, Sichuan, P.R. China; Department of Laboratory Medicine, North Sichuan Medical College, Nanchong, Sichuan, P.R. China; and Translational Medicine Research Center, North Sichuan Medical College, Nanchong, Sichuan, P.R. China
Xiuping Duan, Sichuan Key Laboratory of Medical Imaging, Department of Radiology, Affiliated Hospital of North Sichuan Medical College, Nanchong, Sichuan, P.R. China

overall as well as diagnosing early-stage HCC [17, 18]. Moreover, AFP is also expressed in cases involving benign liver diseases (BLDs), such as cirrhosis and chronic hepatitis B (CHB) and C (CHC), which complicates the differential diagnosis of HCC vs. BLD [19, 20]. As a newly discovered tumour marker in HCC diagnosis, PIVKA-II has a higher diagnostic value than AFP, with a sensitivity for CHB-related early-stage HCC that exceeds 50 % and a specificity of approximately 90 % [21]. Li et al. analysed the data from 49 articles, including 14,118 cases of HCC and 1,544 cases of early HCC. They demonstrated that the sensitivity levels for AFP and PIVKA-II were only 59 and 63 %, respectively. The specificity percentages were only 86 and 91 % for HCC diagnosis, while the sensitivities were only 48 and 45 %, and the specificities were only 89 and 95 %, respectively, for early-stage HCC diagnosis [22].

Bilirubin is the final decomposition product of haemoglobin, a diagnostic marker of liver disease and haematological disease. Because of its antioxidant effect, bilirubin is widely believed to be a protective factor for inflammation, diabetes, cardiovascular disease, metabolic syndrome, and chronic liver disease. Mild unconjugated hyperbilirubinemia may play a role in preventing cardiovascular diseases and cancer [23–25]. Several studies have shown that the serum level of bilirubin in HCC patients is far lower than that in BLD patients [19, 26].

Accordingly, we hypothesized that bilirubin could be used as a biomarker in the differential diagnosis of HCC from BLD. We investigated whether a mathematical model incorporating PIVKA-II, AFP, and bilirubin could improve the efficiency of HCC diagnosis, especially early-stage HCC diagnosis, and the differential diagnosis of HCC from BLD.

Materials and methods

Study setting and patients

A total of 3,481 patients, including 718 HCC patients and 2,763 BLD patients, were retrospectively enrolled at the Affiliated Hospital of Northern Sichuan Medical College from May 2016 to November 2020. The diagnostic criteria for HCC followed the guidelines for the diagnosis and treatment of primary HCC issued by the Chinese Society of Clinical Oncology (2018. V1) [14]. The 2,763 BLD patients who were enrolled included 986 patients with CHB, three patients with CHC, 541 patients with hepatitis B or C-related cirrhosis, 16 patients with alcoholic hepatitis, 90 patients with alcoholic cirrhosis, 80 patients with drug-induced hepatitis, 43 patients with drug-induced cirrhosis, 245 patients with calculous cholangitis, 411 patients with calculous cholecystitis, 39 patients with gallbladder polyps, six patients with liver tuberculosis, 67 patients with a liver cyst, 38 patients with liver abscesses, two patients with liver leiomyosarcoma, 135 patients with liver haemangioma, 14 patients with fatty liver, 34 patients with primary biliary cirrhosis, eight patients with autoimmune hepatitis and five patients with primary biliary cholangitis. All the BLD patients were followed up for at least six months, and none developed HCC during the follow-up period. Before their blood samples were collected, none of the HCC or BLD patients received any clinical treatment related to HCC and BLD.

Detecting serum levels of PIVKA-II, AFP, and bilirubin

Serum levels of PIVKA-II were detected by chemiluminescent microparticle immunoassay (Architect i1000, Abbott Laboratories, Chicago, IL, USA), and serum levels of AFP were detected by electrochemiluminescence immunoassay (Cobas E602, Roche, Inc., Mannheim, Germany). Serum levels of bilirubin were detected by biochemical rate assay (AU5800, Beckman Coulter, Inc., Brea, CA, USA).

Data processing mode for the combined application of PIVKA-II and AFP

The cut-off value for PIVKA-II and AFP in diagnosing HCC was determined using receiver operating characteristic (ROC) curves. The multiple serum levels of PIVKA-II and AFP relative to their corresponding cut-off value were expressed by $M_{cut\text{-}off.}$ This study evaluated the performance of PIVKA-II combined with AFP (PA combination) in the diagnosis of HCC by analysing the $M_{cut\text{-}off}$ sums of PIVKA-II and AFP [27, 28].

Statistical analysis

Data are expressed as the median (interquartile range) or the number (%). The mathematical model of PIVKA-II^2 × AFP/Bilirubin^3 was used as the combined diagnostic model, incorporating PIVKA-II, AFP, and bilirubin (PAB combination) in the diagnosis of HCC, and the method for establishing mathematical model was based on the fact that the serum levels of PIVKA-IIand AFP in HCC patients were higher than those of BLD patients, while the serum levels of bilirubin were lower than those of BLD patients. Pearson's chi-square test was used to compare the enumeration data between two or multiple groups. The Mann–Whitney U test was used to compare measurement data between the two groups. Pearson's correlation analysis was used for two-factor correlation analysis. ROC curves were used to determine the cut-off values, the area under the ROC curves (AUC_S), sensitivity, and specificity of different diagnostic models in the diagnosis of HCC. Statistical analyses were performed using SPSS version 19.0 (IBM SPSS Inc., Armonk, NY, USA) and MedCalc version 12.3 (MedCalc Software bvba, Ostend, Belgium). The statistical significance of all the tests was defined as $p<0.05$ based on two-tailed tests.

Results

Patient characteristics

The HCC and BLD patients were predominantly middle-aged or older and male. The median age of the HCC patients was 58 (49–67) years old, which was significantly higher than that of the BLD patients (52 [44–62] years old) ($p<0.001$). The serum levels of PIVKA-II, AFP, and the PAB combination in HCC patients were higher than those in BLD patients ($p<0.001$), while the serum levels of bilirubin in HCC patients were

lower than those in BLD patients (p<0.001). The proportion of HCC patients with serum levels of PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL was higher than that in BLD patients (p<0.001). The serum levels of PIVKA-II, AFP, and PAB in combination were positively correlated with tumour size (r=0.456, 0.315, and 0.150; p<0.001) (Figure 1). The correlation

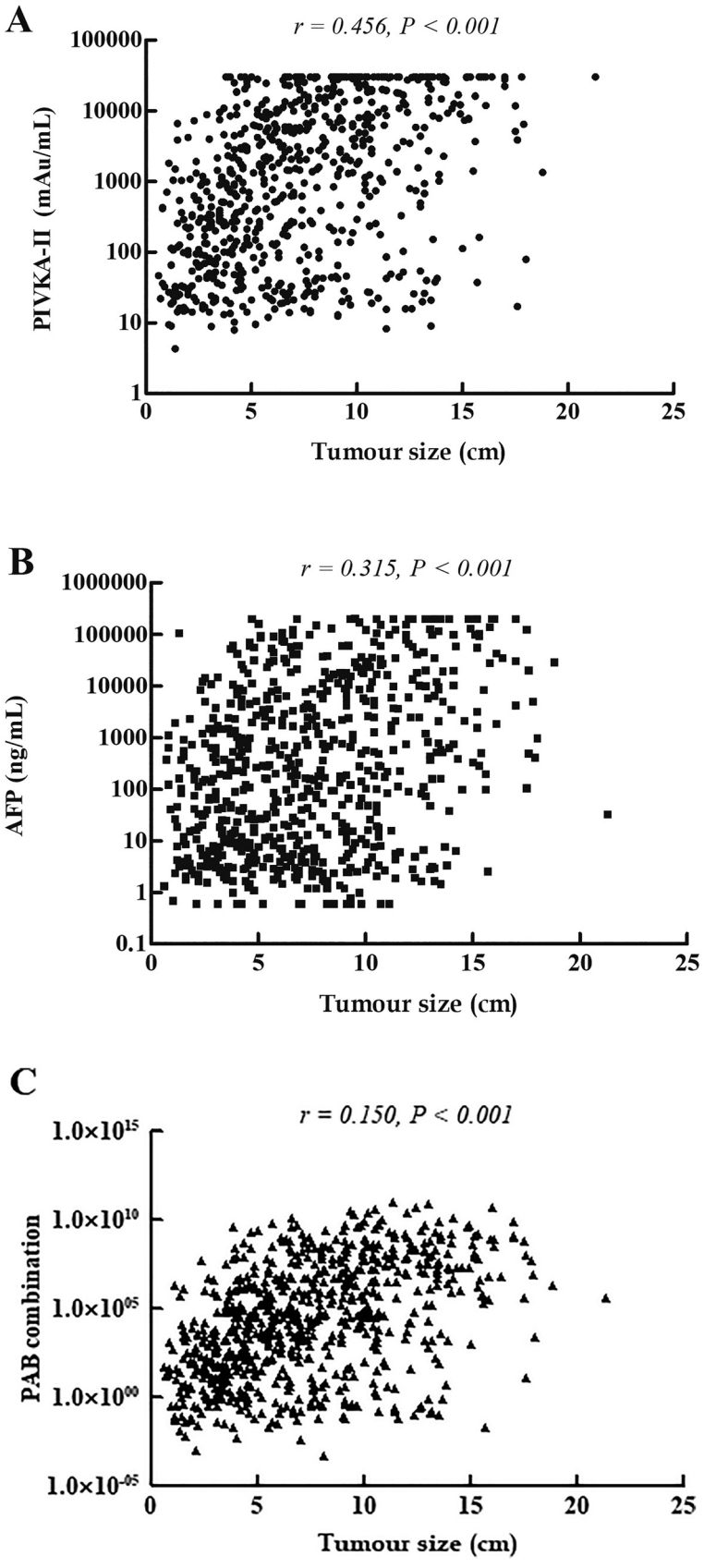

Figure 1: Correlations of serum levels of PIVKA-II (A), AFP (B), and the PAB combination (C) with tumour size in HCC patients.

Table 1: Clinical characteristics of the 3,481 patients.

Characteristics	HCC (n=718)	BLD (n=2,763)	p-Value
Age, years	58 (49–67)	52 (44–62)	<0.001
Sex (male:female)	595:123	1,753:1,010	<0.001
PIVKA-II, mAU/mL	1,280.53 (102.57–10449.65)	24.52 (18.59–35.00)	<0.001
AFP, ng/mL	178.20 (7.98–5,123.78)	3.60 (1.90–9.10)	<0.001
Bilirubin, μmol/L	21.05 (15.20–31.70)	24.50 (15.10–84.10)	<0.001
PAB combination (log)	4.29 (1.65–6.78)	−0.82 (−1.77 to −0.18)	<0.001
PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL, n (%)	646 (89.97 %)	906 (32.79 %)	<0.001
Tumour size, cm	6.80 (4.18–10.10)	NA	NA
HCC<3 cm, n (%)	95 (13.23 %)	NA	NA

Data are expressed as median (interquartile range) or number (%); NA, not applicable; PIVKA-II, protein induced by vitamin K absence or antagonist-II; AFP, alpha-fetoprotein; PAB combination, PIVKA-II, and AFP combined with bilirubin; HCC, hepatocellular carcinoma; BLD, benign liver disease.

between the PAB combination and tumour size was significantly weaker than that of PIVKA-II and AFP (p<0.001). The clinical characteristics of the 3,481 patients are shown in Table 1.

Expression of bilirubin in HCC and BLD patients

Table 2 shows the proportion of cases with serum levels of bilirubin≥20.0 μmol/L, ≥50.0 μmol/L, ≥100.0 μmol/L, and ≥200.0 μmol/L in the BLD and HCC groups and the corresponding positive predictive value (PPV) and negative predictive value (NPV). The proportions of cases with these different serum bilirubin levels were higher in the BLD group than in the HCC group (all p<0.05). The proportions of cases in the BLD group with these different serum bilirubin levels who also had serum levels of PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL were significantly higher than those in the total BLD group for the corresponding serum bilirubin levels (all p<0.001). The proportions of cases in the HCC group with serum bilirubin levels of ≥20.0 μmol/L, ≥100.0 μmol/L, and ≥200.0 μmol/L who also had serum levels of PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL were not significantly different from that of the total HCC group for the corresponding serum bilirubin levels (all p>0.05). However, the proportion of HCC cases with serum bilirubin levels of ≥50.00 μmol/L who also had serum PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL was different from that in the total HCC group for this serum bilirubin level (p=0.004). The proportions of BLD and HCC cases decreased with increasing serum bilirubin levels (p<0.001). As the serum bilirubin levels increased, the negative predictive value gradually increased, while the positive predictive value gradually decreased (both p<0.001).

Table 2: Expression characteristics of bilirubin in HCC and BLD patients.

Bilirubin	HCC, n (%)	BLD, n (%)	PPV, %	NPV, %
All cases				
≥20.00 μmol/L	388 (54.03 %)	1,628 (58.92 %)	19.25	80.75
≥50.00 μmol/L	125 (11.14 %)	921 (33.33 %)	11.95	88.05
≥100.00 μmol/L	34 (4.74 %)	640 (23.16 %)	5.04	94.96
≥200.00 μmol/L	16 (2.23 %)	389 (14.08 %)	3.95	96.05
Cases with PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL				
≥20.00 μmol/L	360 (55.73 %)	788 (86.98 %)	31.36	68.64
≥50.00 μmol/L	77 (13.53 %)	620 (68.43 %)	11.05	88.95
≥100.00 μmol/L	34 (5.26 %)	487 (53.75 %)	6.53	93.47
≥200.00 μmol/L	16 (2.48 %)	316 (34.88 %)	4.82	95.18

Data are expressed as numbers (%). PIVKA-II, protein induced by vitamin K absence or antagonist-II; AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma; BLD, benign liver disease; PPV, positive predictive value; NPV, negative predictive value.

Serum levels of PIVKA-II and AFP correlated positively with serum levels of bilirubin in BLD patients (r=0.107, p<0.001; r=0.167, p<0.001); their correlation coefficients were slightly higher than those in HCC patients (r=0.084, p=0.025; r=0.120, p=0.001) (Figure 2), although these differences were not statistically significant (p>0.05)

Performance of the PAB combination in diagnosing HCC

When using BLD cases as the control group, the AUC of the PA combination for diagnosing HCC was 0.883 (95 % CI: 0.867–0.899), which was significantly higher than that of AFP (0.815: 0.795–0.834) (p<0.001) but not significantly different from that of PIVKA-II (0.883 vs. 0.884) (p=0.934) (Figure 3A).

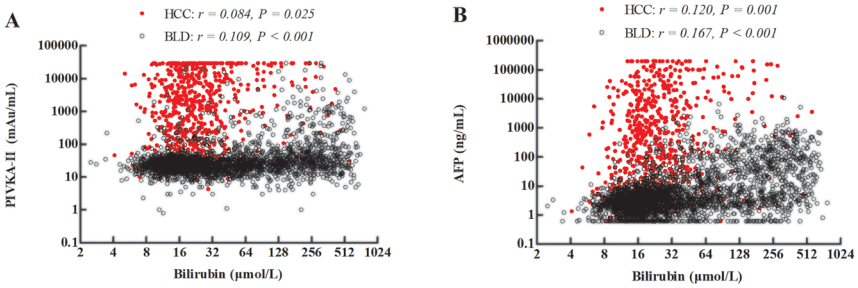

Figure 2: Correlations of serum levels of PIVKA-II (A) and AFP (B) with bilirubin in HCC and BLD patients.

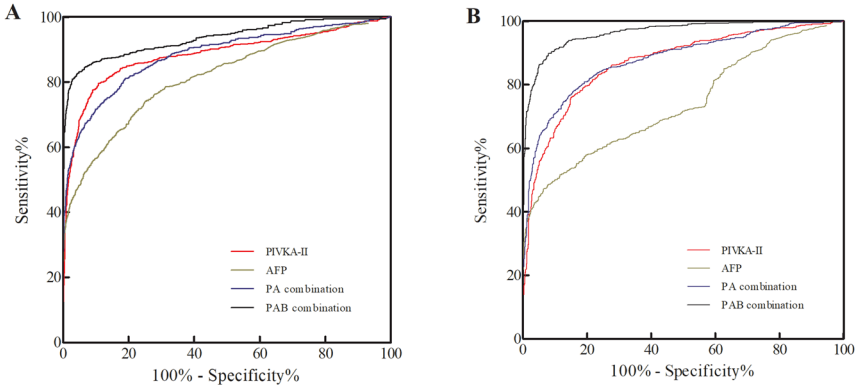

Figure 3: ROC curves of PIVKA-II, AFP, the PA combination, and the PAB combination in HCC patients (A) and HCC patients with serum PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL (B).

Table 3: Performance value of different diagnostic models in diagnosing HCC.

Parameter	PIVKA–II, mAU/mL	AFP, ng/mL	PA combination	PAB combination
HCC				
Cut-off	64.46	9.05	4.41	5.90
AUC	0.884	0.815	0.883	0.935
(95 % CI)	(0.866–0.901)	(0.795–0.834)	(0.867–0.899)	(0.923–0.947)
Sensitivity, %	79.67	74.09	80.92	82.45
Specificity, %	89.03	74.99	81.18	95.77
PPV, %	65.37	43.50	52.77	83.51
NPV, %	94.40	91.76	94.24	95.45
HCC (PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL)				
Cut-off	221.93	406.75	1.90	27.97
AUC	0.870	0.738	0.879	0.965
(95 % CI)	(0.851–0.888)	(0.712–0.764)	(0.861–0.897)	(0.956–0.973)
Sensitivity, %	76.16	48.45	77.55	86.38
Specificity, %	84.77	92.38	84.66	94.72
PPV, %	78.10	81.93	78.28	92.38
NPV, %	83.30	71.54	84.10	90.72

PIVKA-II, protein induced by vitamin K absence or antagonist-II; AFP, alpha-fetoprotein; PA combination, PIVKA-II combined with AFP; PAB combination, PIVKA-II, and AFP combined with bilirubin; AUC, the area under the receiver operating characteristic curve; CI, confidence interval; HCC, hepatocellular carcinoma; PPV, positive predictive value; NPV, negative predictive value.

When using the PAB combination in the diagnosis of HCC, the AUC increased to 0.935 (95 % CI: 0.923–0.947) (Figure 3A), which was significantly higher than that of the PA combination (p<0.001). The sensitivity, specificity, PPV, and NPV of the PAB combination for HCC diagnosis were 82.45 , 95.77, 83.51, and 95.45 %, respectively, which were better than those of the PIVKA-II, AFP, and PA combination (Table 3).

There were 906 cases of BLD and 646 cases of HCC with serum levels of PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL. BLD cases with serum PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL as the control group, the AUCs of PIVKA-II, AFP, and PA combination in diagnosing HCC with serum PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL were 0.870 (95 % CI: 0.851–0.888), 0.738 (95 % CI: 0.712–0.764), and 0.879 (95 % CI: 0.861–0.897) (Figure 3B), which were slightly decreased compared to the HCC values. The AUC of the PAB combination in the diagnosis of HCC with serum levels of PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL was further increased to 0.965 (95 % CI: 0.956–0.973) (Figure 3B), which was significantly higher than that of the PA combination (p<0.001). The sensitivity, specificity, PPV, and NPV of the PAB combination for diagnosing HCC with serum PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL were 86.38 , 94.92, 92.38, and 90.72 %, respectively, which were better than those of PIVKA-II alone, AFP alone, and the PA combination (Table 3).

Performance value of the PAB combination in diagnosing HCC<3.0 cm

There were 95 cases of HCC with tumour sizes<3.0 cm (HCC<3.0 cm). In the 2,763 BLD cases used as the control group, the AUC of the PA combination in the diagnosis of HCC<3.0 cm was 0.808 (95 % confidence interval (CI): 0.763–0.853), which was slightly higher than that of PIVKA-II (0.756 [95 % CI: 0.696–0.817]) and AFP (0.749 [95 % CI: 0.697–0.801]), but without a statistically significant difference (p=0.178 and p=0.096) (Figure 4A). The AUC of the PAB combination for diagnosing HCC<3.0 cm was 0.862 (95 % CI: 0.815–0.910), which was slightly higher than that of the PA combination but without a statistically significant difference (p=0.104) (Figure 4A). The sensitivity, specificity, PPV, and NPV of the PAB combination in diagnosing HCC<3.0 cm were 71.58 , 93.45, 27.31, and 98.97 %, respectively. Except that the sensitivity of the PAB combination was lower than that of the PA combination (81.05 %), the other diagnostic performance parameter values were better for the PAB combination than for the PIVKA-II, AFP, and PA combination (Table 4).

There were 80 cases of HCC<3.0 cm with serum levels of PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL. For 906 BLD cases with serum levels of PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL as the control group, the AUCs of PIVKA-II, AFP, and PA combination used in the diagnosis of HCC<3.0 cm were 0.695 (95 % CI: 0.630–0.759), 0.636 (95 % CI: 0.570–0.703), and 0.732 (95 % CI: 0.673–0.790), respectively (Figure 4B). The AUC of the PAB combination for diagnosing HCC<3.0 cm with serum PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL was 0.910 (95 % CI: 0.878–0.943), which was higher than that of the PA combination (p<0.001) (Figure 4B). The sensitivity, specificity, PPV, and NPV of the PAB combination used in the diagnosis of HCC<3.0 cm with serum levels of PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL were 86.25 ,

Table 4: Performance value of different diagnostic models in diagnosing HCC<3.0 cm.

Parameter	PIVKA–II, mAU/mL	AFP, ng/mL	PA combination	PAB combination
HCC<3.0 cm				
Cut-off	62.34	10.95	1.63	3.37
AUC	0.756	0.749	0.808	0.862
(95 % CI)	(0.696–0.817)	(0.697–0.801)	(0.763–0.853)	(0.815–0.910)
Sensitivity, %	61.05	62.11	81.05	71.58
Specificity, %	88.60	77.05	71.73	93.45
PPV, %	15.55	8.51	8.97	27.31
NPV, %	98.51	98.34	99.10	98.97
HCC<3.0 cm (PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL)				
Cut-off	89.79	373.70	1.11	2.56
AUC	0.695	0.738	0.732	0.910
(95 % CI)	(0.630–0.759)	(0.570–0.703)	(0.673–0.790)	(0.878–0.943)
Sensitivity, %	66.25	31.25	78.75	86.25
Specificity, %	73.29	91.06	63.02	85.10
PPV, %	17.97	23.59	15.83	33.82
NPV, %	96.09	93.75	97.11	98.59

PIVKA-II, protein induced by vitamin K absence or antagonist-II; AFP, alpha-fetoprotein; PA combination, PIVKA-II combined with AFP; PAB combination, PIVKA-II, and AFP combined with Bilirubin; AUC, the area under the receiver operating characteristic curve; CI, confidence interval; HCC, hepatocellular carcinoma; PPV, positive predictive value; NPV, negative predictive value.

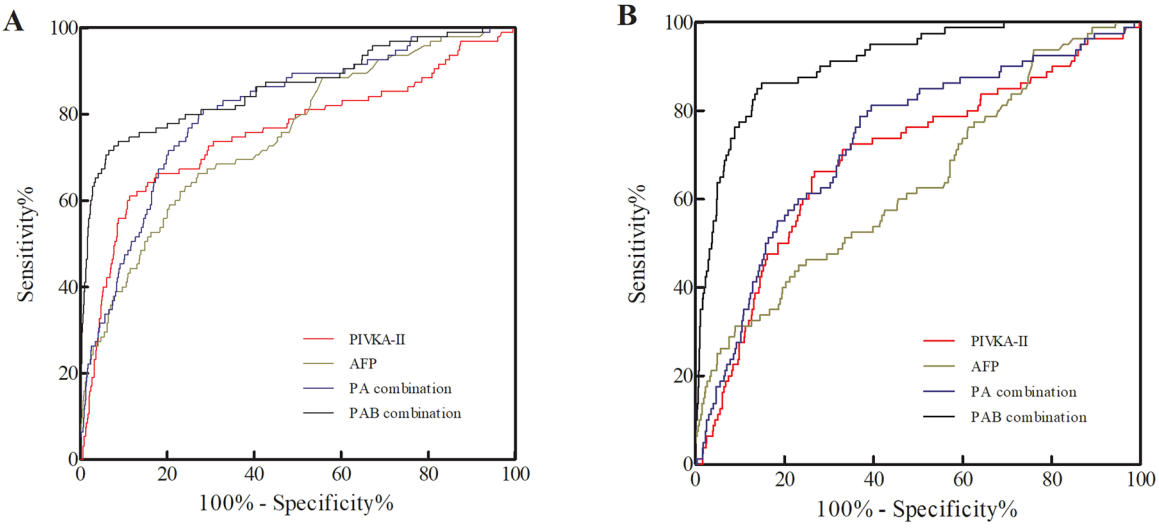

Figure 4: ROC curves of PIVKA-II, AFP, the PA combination, and the PAB combination in HCC<3.0 cm patients (A) and HCC<3.0 cm patients with serum levels of PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL (B).

85.10, 33.82, and 98.59 %, respectively. Except that the specificity of the PAB combination was lower than that of AFP (91.06 %), the other diagnostic performance parameter values of the PAB combination were better than those of the PIVKA-II, AFP, and PA combination (Table 4).

Discussion

This study established a combined mathematical diagnostic model by incorporating the levels of PIVKA-II and AFP combined with the bilirubin levels for HCC diagnosis. The ROC curve analysis showed that the performance of the PAB combination in the diagnosis of HCC and early-stage HCC was better than that of the PIVKA-II, AFP, or PA combination, with sensitivity exceeding 70 % and specificity exceeding 90 %, which was better than our previous research data [27]. Moreover, the performance of the PAB combination in the diagnosis of HCC and early-stage HCC with serum levels of PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL was further improved. These results indicated that bilirubin was a superior biomarker for diagnosing HCC and for its differential diagnosis from BLD. Bilirubin combined with PIVKA-II and AFP in the diagnosis of HCC can improve the probability of detecting HCC at an early stage and reduce the occurrence of missed diagnosis and misdiagnosis to improve the survival time and quality of life of HCC patients.

Bilirubin is considered a protective factor for BLD, and it plays a role in preventing tumorigenesis [23–25]. The data in this study showed that the serum levels of bilirubin in BLD cases were significantly higher than those in HCC cases. The proportion of elevated serum bilirubin in BLD cases was significantly higher than that in HCC cases, which was consistent with existing research reports [19, 26]. Interestingly, the correlations between serum PIVKA-II, AFP, and bilirubin in BLD cases were slightly stronger than those in HCC cases. Additionally, among BLD and HCC patients with serum PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL, the proportions of BLD cases with high bilirubin were significantly higher than those of HCC cases. It is unclear whether this observation reflects the initial liver compensation mechanism in BLD patients and a hepatoprotective measure, which is worth further study. PIVKA-II and AFP are highly expressed in some BLD cases, which is essential in the difficulty of making a differential diagnosis between HCC and BLD [19, 20, 29]. As shown in Table 2, with the increase in serum bilirubin levels, the PPV of bilirubin in diagnosing HCC decreased gradually, and the NPV increased gradually, indicating that bilirubin can be used as a biomarker in the differential diagnosis of HCC from BLD; a higher serum bilirubin level indicates a lower probability of HCC and a higher probability of BLD. However, because of the poor performance of bilirubin in diagnosing HCC, and based on the characteristics of the difference in bilirubin serum levels between BLD patients and HCC patients, we established a mathematical model in which bilirubin is combined with PIVKA-II and AFP for HCC diagnosis and differential diagnosis of HCC from BLD. The sensitivity and specificity of the PAB combination in diagnosing HCC and HCC<3.0 cm were significantly improved, indicating that the PAB combination could facilitate HCC diagnosis and the differential diagnosis of HCC from BLD. As shown in Figure 1, the correlation between the PAB combination and the tumour size was markedly weaker than that of PIVKA-II and AFP, indicating that the PAB combination was more suitable than PIVKA-II and AFP in diagnosing early-stage HCC.

PIVKA-II≥40 mAU/mL and AFP≥20 ng/mL are often used as cut-off values in HCC diagnosis [30–32]. Generally, before the appearance of clinical symptoms, abnormal PIVKA-II or AFP levels should attract the attention of clinicians and patients. Moreover, HCC and HCC<3 cm cases with PIVKA-II and/or AFP levels account for more than 80 % of all HCC and of all HCC<3.0 cm cases, respectively. Therefore, we evaluated the performance of the PAB combination in diagnosing HCC and HCC<3.0 cm cases in those with serum PIVKA-II≥40 mAU/mL and/or AFP≥20 ng/mL. In these HCC and HCC<3.0 cm cases, the performance parameter values of the PIVKA-II, AFP, and PA combination decreased, while the corresponding values of the PAB combination improved. The results indicated that PIVKA-II, AFP, and PA combinations were less effective in differentiating between BLD and HCC, particularly early-stage HCC, in which the serum levels of PIVKA-II and AFP are far lower than those in the middle and late stages of HCC [27], which complicates the differential diagnosis. The PAB combination will thus be more suitable for diagnosing HCC with abnormal PIVKA-II and AFP results.

Undeniably, this study had some limitations. The PPV of the PAB combination for diagnosing HCC<3.0 cm was only approximately 30 %, which may be related to the small number of HCC<3.0 cm cases (accounting for only 13.23 % of all HCC cases from the same period).

Conclusions

We derived a model in which HCC can be diagnosed earlier and distinguished from BLD. Follow-up monitoring of PIVKA-II, AFP, and bilirubin should be strengthened in high-risk populations, such as patients with CHB infection or liver cirrhosis, to improve the diagnosis and treatment of HCC at

an earlier stage and thereby improve the survival rate, prognosis, and quality of life of HCC patients. Meanwhile, as a superior biomarker for diagnosing HCC and distinguishing HCC from BLD, the level of bilirubin should be observed when abnormal AFP and/or PIVKA-II were found, and the higher level of bilirubin indicated the higher possibility of BLD, and the lower level of bilirubin indicated the higher possibility of HCC.

Research ethics: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013). The conduct of this study was approved by the Ethics Committee of the Affiliated Hospital of North Sichuan Medical College (2022ER184-1).
Informed consent: Informed consent was obtained from all individuals included in this study.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Competing interests: Authors state no conflict of interest.
Research funding: This work was supported by the Science and Technology Project of Nanchong (20SXQT0337) and the Nanchong City's S&T strategic cooperation projects between the city and the university (19SXHZ0274).

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359–86.
2. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, et al. Cancer statistics in China, 2015. CA Cancer J Clin 2016;66:115–32.
3. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics. CA Cancer J Clin 2015;65:87–108.
4. Baecker A, Liu X, La Vecchia C, Zhang ZF. Worldwide incidence of hepatocellular carcinoma cases attributable to major risk factors. Eur J Cancer Prev 2018;27:205–12.
5. de Martel C, Maucort-Boulch D, Plummer M, Franceschi S. Worldwide relative contribution of hepatitis B and C viruses in hepatocellular carcinoma. Hepatology 2015;62:1190–200.
6. El-Serag HB. Hepatocellular carcinoma. N Engl J Med 2011;365:1118–27.
7. Everhart JE, Ruhl CE. Burden of digestive diseases in the United States Part III: liver, biliary tract, and pancreas. Gastroenterology 2009;136: 1134–44.
8. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008; 359:378–90.
9. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet 2003; 362:1907–17.
10. Bruix J, Reig M, Sherman M. Evidence-based diagnosis, staging, and treatment of patients with hepatocellular carcinoma. Gastroenterology 2016;150:835–53.
11. Yu R, Tan Z, Xiang X, Dan Y, Deng G. Effectiveness of PIVKA-II in the detection of hepatocellular carcinoma based on real-world clinical data. BMC Cancer 2017;17:608.
12. Grandhi MS, Kim AK, Ronnekleiv-Kelly SM, Kamel IR, Ghasebeh MA, Pawlik TM. Hepatocellular carcinoma: from diagnosis to treatment. Surg Oncol 2016;25:74–85.
13. Kim SH, Moon DB, Kim WJ, Kang WH, Kwon JH, Jwa EK, et al. Preoperative prognostic values of α-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist-II (PIVKA-II) in patients with hepatocellular carcinoma for living donor liver transplantation. Hepatobiliary Surg Nutr 2016;5:461–9.
14. Chinese Society of Clinical Oncology. Guidelines of Chinese society of clinical Oncology (CSCO) hepatocellular carcinoma (in Chinese). Beijing: People's Medical Publishing House; 2018.
15. Kudo M, Izumi N, Kokudo N, Matsui O, Sakamoto M, Nakashima O, et al. Management of hepatocellular carcinoma in Japan: consensus-based clinical practice guidelines proposed by the Japan society of hepatology (JSH) 2010 updated version. Dig Dis 2011;29:339–64.
16. Izumi N. Diagnostic and treatment algorithm of the Japanese society of hepatology: a consensus-based practice guideline. Oncology 2010;78: 78–86.
17. Ertle JM, Heider D, Wichert M, Keller B, Kueper R, Hilgard P, et al. A combination of α-fetoprotein and des-γ-carboxy prothrombin is superior in detection of hepatocellular carcinoma. Digestion 2013;87:121–31.
18. Reichl P, Mikulits W. Accuracy of novel diagnostic biomarkers for hepatocellular carcinoma: an update for clinicians (Review). Oncol Rep 2016;36:613–25.
19. Park SJ, Jang JY, Jeong SW, Cho YK, Lee SH, Kim SG, et al. Usefulness of AFP, AFP-L3, and PIVKA-II, and their combinations in diagnosing hepatocellular carcinoma. Medicine 2017;96:e5811.
20. Forner A, Bruix J. Biomarkers for early diagnosis of hepatocellular carcinoma. Lancet Oncol 2012;13:750–1.
21. Gao J, Song P. Combination of triple biomarkers AFP, AFP-L3, and PIVAKII for early detection of hepatocellular carcinoma in China: expectation. Drug Discov Ther 2017;11:168–9.
22. Li C, Zhang Z, Zhang P, Liu J. Diagnostic accuracy of des-gamma-carboxy prothrombin versus α-fetoprotein for hepatocellular carcinoma: a systematic review. Hepatol Res 2014;44:E11–25.
23. Fevery J. Bilirubin in clinical practice: a review. Liver Int 2008;28: 592–605.
24. Seyed Khoei N, Jenab M, Murphy N, Banbury BL, Carreras-Torres R, Viallon V, et al. Circulating bilirubin levels and risk of colorectal cancer: serological and Mendelian randomization analyses. BMC Med 2020;18:229.
25. Weaver L, Hamoud AR, Stec DE, Hinds TD Jr. Biliverdin reductase and bilirubin in hepatic disease. Am J Physiol Gastrointest Liver Physiol 2018;314:G668–76.
26. Wang T, Zhang KH, Hu PP, Wan QS, Han FL, Zhou JM, et al. Combination of dual serum fluorescence, AFP and hepatic function tests is valuable to identify HCC in AFP-elevated liver diseases. Oncotarget 2017;8: 97758–68.
27. Wang Q, Chen Q, Zhang X, Lu XL, Du Q, Zhu T, et al. Diagnostic value of gamma-glutamyltransferase/aspartate aminotransferase ratio, protein induced by vitamin K absence or antagonist II, and alpha-fetoprotein in hepatitis B virus-related hepatocellular carcinoma. World J Gastroenterol 2019;25:5515–29.
28. Wang G, Lu X, Du Q, Zhang G, Wang D, Wang Q, et al. Diagnostic value of the γ-glutamyltransferase and alanine transaminase ratio, alpha-fetoprotein, and protein induced by vitamin K absence or antagonist II in hepatitis B virus-related hepatocellular carcinoma. Sci Rep 2020;10: 13519.

29. Feng H, Li B, Li Z, Wei Q, Ren L. PIVKA-II serves as a potential biomarker that complements AFP for the diagnosis of hepatocellular carcinoma. BMC Cancer 2021;21:401.
30. Park H, Park JY. Clinical significance of AFP and PIVKA-II responses for monitoring treatment outcomes and predicting prognosis in patients with hepatocellular carcinoma. BioMed Res Int 2013;2013:310427.
31. Xu F, Zhang L, He W, Song D, Ji X, Shao J. The diagnostic value of serum PIVKA-II alone or in combination with AFP in Chinese hepatocellular carcinoma patients. Dis Markers 2021;2021:8868370.
32. Kim KH, Kim JY, Yoo JS. Mass spectrometry analysis of glycoprotein biomarkers in human blood of hepatocellular carcinoma. Expert Rev Proteomics 2019;16:553–68.