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Original Article

Diagnostic Efficacy and Possible Underlying Mechanisms of Noninvasive Clinical Markers in Hepatocellular Carcinoma

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Abstract

Background and Aims: In this study, we aimed to evaluate the diagnostic values of alpha-fetoprotein (AFP), soluble AXL (sAXL), des-γ-carboxy prothrombin (DCP), the aspartate aminotransferase-to-platelet ratio index (APRI), and the gamma-glutamyl transpeptidase-to-platelet ratio (GPR) in hepatocellular carcinoma (HCC) and the possible underlying mechanisms of the correlations between them. ***Methods:*** We collected serum samples from 190, 128, and 75 patients with HCC, cirrhosis, and chronic viral hepatitis, and from 82 healthy subjects. Serum levels of AFP, sAXL, and DCP were determined, and APRI and GPR values were calculated. Receiver operating characteristic (ROC) curves were used to analyze the diagnostic value of single and combined biomarkers. ***Results:*** We detected significant differences between the HCC group and other groups regarding serum AFP, sAXL, DCP, and APRI levels. GPR significantly differed between the HCC group and other groups, except for the liver cirrhosis group. AFP, sAXL, DCP, APRI, and GPR had positive correlations with each other, and AFP showed a higher area under the curve (AUC) and Youden index values, while APRI and DCP showed the highest sensitivity and specificity. Also, when AFP was combined with sAXL, DCP, APRI, and GRP, the highest AUC (0.911) and a higher net reclassification improvement value were obtained compared with those obtained for the individual biomarkers. ***Conclusions:*** AFP, sAXL, DCP, APRI, and GPR are independent risk factors for HCC, and the diagnostic performance of AFP combined with sAXL, DCP, APRI, and GPR for HCC diagnosis was superior to that of the individual biomarkers.

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Keywords: Hepatocellular carcinoma; HCC-specific biomarkers; Alpha-fetoprotein; Chronic viral hepatitis Cirrhosis.

Abbreviations: AFP, alpha-fetoprotein; APRI, aminotransferase-to-platelet ratio index; AUC, area under the curve; c-Met, cellular mesenchymal-epithelial transition factor; DCP, des-γ-carboxy prothrombin; GPR, gamma-glutamyl transpeptidase-to-platelet ratio; HC, healthy controls; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; LC, liver cirrhosis; ROC, Receiver operating characteristic; sAXL, soluble AXL; VH, viral hepatitis.

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Introduction

Hepatocellular carcinoma (HCC) is a highly malignant cancer, and unfortunately, its early symptoms are difficult to detect. Further, it progresses rapidly and has a low 5-year overall survival rate.[1] Therefore, to improve its prognosis, early diagnostic methods with high sensitivity and good specificity are important. Abdominal ultrasonography, liver biopsy, magnetic resonance imaging, using noninvasive markers, and computed tomography are the main methods for diagnosing liver cancer.[2] To ensure patient safety and reduce invasive examinations and the impact of computed tomography on the body, recent clinical studies have focused on noninvasive markers for HCC diagnosis.[3]

Early diagnosis of HCC based on the levels of serum alpha-fetoprotein (AFP) is widely accepted by the international medical community.[4] Studies have also shown that about 50% of patients with HCC, especially those with early and small HCCs, are AFP negative. Novel biomarkers and related scoring indicators have been proposed to complement AFP and improve the accuracy of HCC diagnosis.[5,6] However, the clinical value of those markers is debatable and determining their application values is challenging. Further in-depth studies are needed improve HCC diagnosis.

The TAM receptor family of receptor tyrosine kinases comprises TYRO3, AXL, and MER, and specifically, AXL is mainly involved in regulating platelet aggregation and maintaining vascular integrity.[7] The upstream regulator of AXL, RAB10, is associated with an advanced stage and a large tumor size in patients with HCC,[8] and following AXL cleavage by a disintegrin and metalloproteinases 10 and 17 via a protein kinase

C-dependent pathway, soluble AXL (sAXL) can be detected in serum.[9]

Des-γ-carboxy prothrombin (DCP) is a prothrombin precursor produced in patients with HCC. It does not interact with other coagulation factors,[10] and many studies have shown that elevated DCP levels are associated with tumors in patients with HCC.[11,12] However, the specific mechanisms underlying this association remain unclear. The aminotransferase-to-platelet ratio index (APRI) is also widely used to noninvasively assess liver fibrosis.[13] A retrospective study revealed that both fibrosis-4 and APRI predicted the risk of liver cancer.[14] Also, the gamma-glutamyl transpeptidase-to-platelet ratio (GPR), which was first proposed by Lemoine *et al*.[15] in 2016, was used by Park *et al*.[16] to predict HCC in Korean patients with chronic hepatitis B.

With the increasing use of noninvasive markers to assess HCC, studies aimed at clarifying the limitations of these markers are required. Therefore, the aim of this study was to analyze the predictive power of noninvasive markers of HCC and the possible mechanisms of the association between them in HCC.

Methods

Patients and serum samples

Serum samples were collected from 190 patients with HCC, 128 patients with liver cirrhosis (LC), 75 patients with chronic viral hepatitis (VH), and 82 healthy controls (HC) between August 2014 and January 2019 at the Phase I Drug Clinical Trial Unit of the Affiliated Hospital of Yanbian University, Yanji, China. Patients with chronic VH had either hepatitis B virus or hepatitis C virus infection. Baseline patient characteristics were collected and recorded. Venous blood samples were also collected from the participants in the morning after fasting for at least 8 h and then sent to the laboratory, where they were frozen and stored until index testing.

HCC and VH were diagnosed according to the guidelines of the American Association for the Study of Liver Diseases and tumor staging was performed based on the Barcelona Clinical Liver Cancer (BCLC) system. Also, cirrhosis was assessed by the investigators based on histological and clinical evidence related to decompensation. The healthy subjects had no family history of liver cancer, no history of liver-related diseases, no abnormal hepatobiliary manifestations, and no abnormal laboratory test results.

Clinical information

Using the electronic medical record system of the Affiliated Hospital of Yanbian University, China, we collected information related to age, sex, ethnicity, and clinical indicators. Data based on imaging examinations of the patients with HCC and cirrhosis were also obtained.

Determination of serum AFP, sAXL, and DCP levels

Serum AFP levels were determined using a chemiluminescent immunoassay analyzer (UniCel DxI 800, Beckman Coulter, Brea, CA, USA) at the Department of Laboratory Medicine, Affiliated Hospital of Yanbian University, Yanji, China. Enzyme-linked immunosorbent assay (ELISA) kits (human AXL DuoSet ELISA, R&D Systems, Minneapolis, MN, USA; human abnormal prothrombin ELISA, Hotgen, Beijing, China) were used to detect sAXL and DCP levels.

Calculation of APRI and GPR indices

APRI was calculated as APRI = 100 × [AST (IU/L)/AST upper limit of normal (ULN)]/[platelet count (PLT) (10^9/L)]. GPR was calculated as GPR = 100 × GGT (IU/L)/PLT (10^9/L)].

Statistical analysis

SPSS software version 26.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis, and $p<0.05$ was considered statistically significant. For multiple comparisons, the Kruskal-Wallis test, which is a nonparametric test, was used, but for bilateral between-group variability analysis, the Mann–Whitney test, which is also a nonparametric test, was performed. Bivariate correlation analysis was used to explore the correlation between the various biomarkers, and binary logistic regression analysis was used to assess the variance of various biomarker combinations. To evaluate the diagnostic value of each biomarker, receiver operating characteristic (ROC) curves were used. The net reclassification improvement (NRI) value was determined using an "extreme smart analysis platform" for analysis and processing.

Results

Clinical characteristics of patients with HCC, LC, VH, and HC

In this study, we enrolled 475 participants. The baseline clinical characteristics are in Table 1 which shows there were no significant differences between the different groups in age and HBV and HCV infections ($p>0.05$). The numbers of men in the HCC, LC, VH, and HC groups were 120 (63.2%), 65 (50.8%), 37 (49.3%), and 29 (35.4%), respectively.

As shown in Figure 1 and Table 1, the median AFP concentration corresponding to the HCC group was 60 ng/mL, which was significantly higher than those in the LC (5.60 ng/mL), VH (2.93 ng/mL), and HC (2.42 ng/mL) groups ($p<0.05$). Further, the median concentration of sAXL for the HCC group (33.55 ng/mL) was significantly higher than those in the LC (29.98 ng/mL), VH (20.82 ng/mL), and HC (11.39 ng/mL) groups ($p<0.05$). The HCC group also had a significantly higher median DCP concentration (40.12 ng/mL) than the LC (9.04 ng/mL), VH (7.84 ng/mL), and HC (4.62 ng/mL) groups ($p<0.05$). However, no significant differences were seen between the LC and VH groups ($p>0.05$). Our results also indicated a significantly higher median APRI concentration in the HCC group (1.21) than in the LC (1.45), VH (0.30), and HC (0.20) groups ($p<0.05$). The HCC group also had a higher median GPR concentration (1.12) than the VH (0.21) and HC (0.11) groups ($p<0.05$). However, there was no significant difference between the HCC and LC groups ($p>0.05$). As shown in Table 2, the median concentration of AFP, sAXL, DCP, APRI, and GPR increased with the BCLC stage of HCC, but the differences were not statistically significant ($p>0.05$).

Key indicators related to HCC

We divided the participants into two groups. One included patients with liver cancer, and the other group included non-HCC patients with cirrhosis or VH and the HCs. To determine the key risk factors involved in the development of HCC, we first performed correlation analysis, which showed that AFP, sAXL, DCP, APRI, and GPR were positively correlated (Fig. 2). The correlations between the biomarkers in all groups were above 0.44, with the strongest correlation seen between APRI and GPR (coefficient=0.81), and the weakest between DCP and GPR (coefficient=0.44). Univariate analysis showed that the odds ratios for age, sex, AFP, sAXL, DCP, APRI, and GPR were all above 1. To reduce the interference of confounding factors, we conducted multivariate analysis to determine the significant risk factors. The results thus obtained were

Table 1. Clinical characteristics of the study participants

Characteristic	HCC	LC	VH	HC	*p*-value
Study participants	190	128	75	82	–
Age	60.37±9.58	58.76±10.60	57.46±12.26	53.46±11.56	>0.05
BCLC stage (0/A/B/C/D)	4/44/76/45/21	–	–	–	–
Male	120 (63.2%)	65 (50.8%)	37 (49.3%)	29 (35.4%)	<0.05
HBV/HCV	81/109	71/57	40/35		>0.05
PLT (*10^9/L)	112.00 (71.25–170.00)	80.00 (56.00–125.50)	197.00 (156.00–254.00)	247.50 (210.00–271.00)	<0.05
AST (U/L)	49.00 (30.00–73.00)	54.00 (35.50–80.50)	25.00 (19.00–36.50)	18.50 (16.00–22.00)	<0.05
ALT (U/L)	36.00 (23.00–55.00)	46.00 (23.50–75.50)	22.00 (17.00–64.50)	14.00 (11.00–21.00)	<0.05
GGT (U/L)	64.50 (38.00–149.00)	47.00 (26.50–102.5)	25.00 (16.00–86.00)	16.00 (13.00–22.00)	<0.05
ALB (g/L)	39.00 (34.00–43.00)	40.00 (32.00–43.00)	45.00 (44.00–48.00)	48.00 (47.00–49.00)	<0.05
TBIL (μmol/L)	18.35 (13.30–26.38)	24.20 (16.60–37.45)	14.60 (12.65–18.95)	12.40 (10.50–15.60)	<0.05
AFP (ng/mL)	60 (6.46–761.75)	5.60 (3.99–8.29)	2.93 (1.99–4.23)	2.42 (1.86–3.26)	<0.05
sAXL (ng/mL)	33.55 (28.79–37.06)	29.98 (26.72–33.68)	20.82 (14.39–28.31)	11.39 (9.87–15.83)	<0.05
DCP (ng/mL)	40.12 (5.76–70.11)	9.04 (5.06–10.66)	7.84 (7.05–11.44)	4.62 (1.88–9.05)	<0.05
GPR	1.12 (0.55–2.29)	0.90 (0.44–1.69)	0.21 (0.14–0.49)	0.11 (0.09–0.17)	<0.05
APRI	1.21 (0.52–2.08)	1.45 (0.71–3.08)	0.30 (0.20–0.63)	0.20 (0.14–0.26)	<0.05

AFP, alpha-fetoprotein; APRI, aminotransferase-to-platelet ratio index; DCP, des-γ-cboxy prothrombin; GPR, gamma-glutamyl transpeptidase-to-platelet ratio; HBV, hepatitis B virus infection; HC, healthy controls; HCC, hepatocellular carcinoma; HCV, hepatitis C virus infection; LC, liver cirrhosis; sAXL, soluble AXL; VH, viral hepatitis.

consistent with those obtained following univariate analysis (p<0.05; Table 3).

Diagnostic accuracy of APRI, GPR, and serum biomarkers for detecting HCC

The area under the ROC curve (AUC), sensitivities, specificities, and optimal threshold values of AFP, sAXL, DCP, APRI, and GPR for the diagnosis of HCC are shown in Table 4. the AUC and Youden's index values corresponding to AFP were higher than those of the other markers. APRI had the highest sensitivity, but DCP showed the highest specificity. We also evaluated the diagnostic value of the combined biomarkers and found that the AUC corresponding to the combined biomarkers was greater than 0.8. Of all the two-marker combinations used for HCC diagnosis, the combination of sAXL with DCP had the highest AUC (0.882), with a higher sensitivity and Youden's index (0.8211 and 0.6648, respectively) compared with those of all the other two-marker combinations. Also, the specificity of AFP combined with DCP was the highest (0.9453) considering all the two-marker combinations. Considering that we evaluated five markers in this study, the significance of analyzing only two-marker combinations could be limited, so we also analyzed three-, four-, and five-marker combinations. The results revealed that among all the three-marker combinations, that of AFP, sAXL, and DCP showed the highest AUC and Youden's index (0.904 and 0.7085, respectively). Further, the combination of sAXL, DCP, and GPR showed the highest sensitivity (0.8418), while the combination of AFP, DCP, and GPR showed the highest specificity (0.9407). We also saw that among all the four-marker combinations, AFP, sAXL, DCP, and APRI showed the highest AUC, Youden's index, and specificity (0.910, 0.7162, and 0.9576, respectively), but the combination of sAXL, DCP, APRI, and GPR had the highest sensitivity (0.8523). Interestingly, the combination of all five markers, AFP, sAXL, DCP, APRI, and GPR, had the highest AUC (0.911), and thus, the greatest diagnostic value for HCC.

We further examined whether multimarker combinations had better diagnostic values than individual markers and whether there were significant differences between the diagnostic values of the different multimarker combinations. First, we compared 26 groups of multimarker combinations with five groups of individual markers. The results are shown in Figure 3 and Table 5. Next, we selected combinations with AUCs >0.9 for comparison with the single group. We saw that four groups of multimarker combinations differed significantly from the five groups of individual markers (p<0.05). However, we observed no significant differences among the four groups of multimarker combinations (p>0.05). Our NRI calculations also indicated that the above multimarker combinations had significantly higher prediction accuracies than the individual markers, and that multimarker combinations were superior to individual markers for diagnosing HCC. Also, the predictive accuracies of AFP, sAXL, DCP, APRI, and GPR in the HCC group were higher than their values for the other three groups, and interestingly, AFP, sAXL, and DCP were all identified as part of the four multimarker combinations with high prediction accuracy.

Discussion

Even though about 50% of patients with HCC are AFP negative, AFP is a first-line clinical biomarker for monitoring and diagnosing HCC.[17] Several biomarkers for HCC diagnosis, such as sAXL, DCP, Golgi protein-73, and lectin-binding AFP-3 have been identified; however, they are still under clinical evaluation.[18,19] Further, even though the pathogenesis of liv-

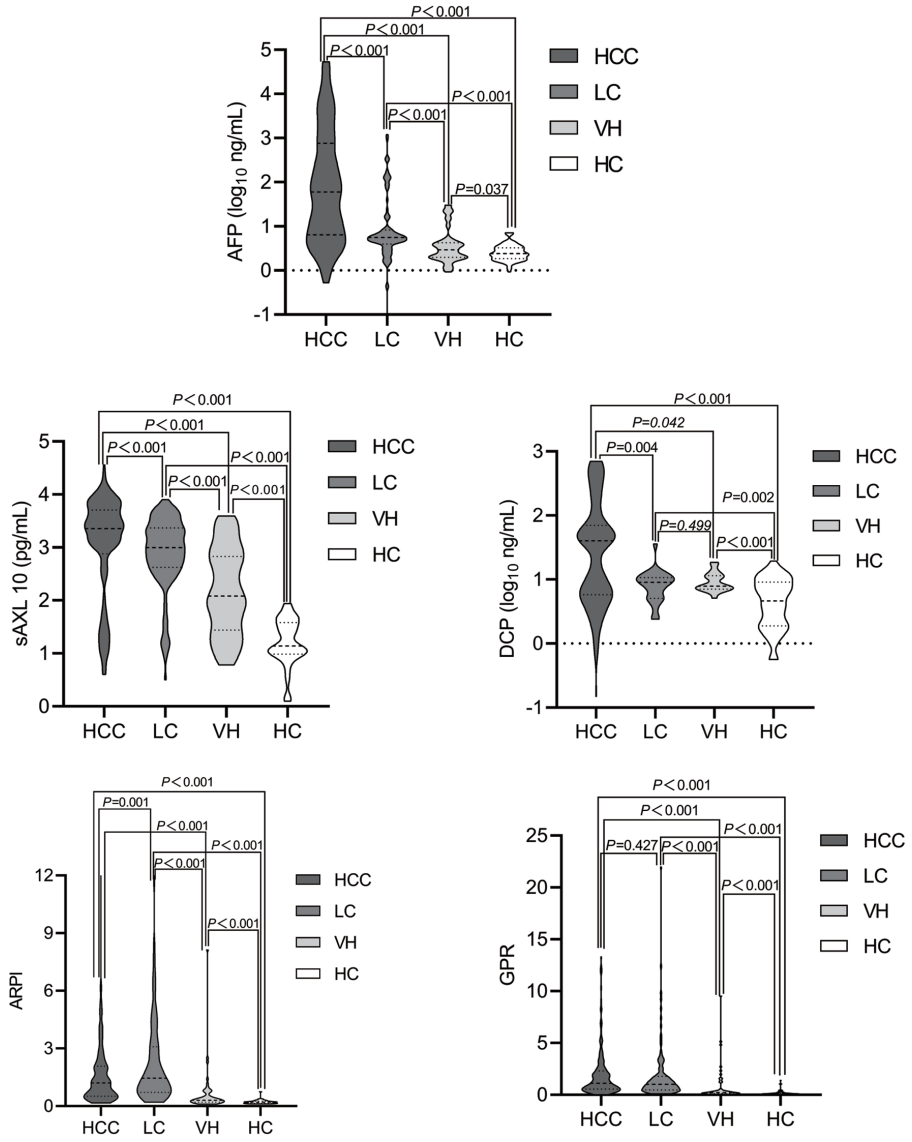

Fig. 1. Distribution of the values of serum biomarkers AFP, sAXL, DCP, APRI, and GPR in the HCC, LC, VH, and HC groups. AFP, alpha-fetoprotein; APRI, aminotransferase-to-platelet ratio index; AUC, area under the receiver operating characteristic curve; CI, confidence interval; DCP, des-γ-carboxy prothrombin; GPR, gamma-glutamyl transpeptidase-to-platelet ratio; HC, healthy control; HCC, hepatocellular carcinoma; LC, liver cirrhosis; sAXL, soluble AXL; VH, viral hepatitis.

er fibrosis-HCC remains unclear, related fibrosis scores, such as APFI, GPR, and fibrosis-4 scores have been widely used to predict HCC development.[20,21] Therefore, we measured and calculated the levels of sAXL, DCP, ARPI, and GPR to test and verify their ability regarding HCC diagnosis. The results showed that using a single marker assay, the diagnostic accuracy of AFP was higher than that of the other detection markers. So AFP remains a reliable and first-line marker for

Table 2. AFP, sAXL, DCP, APRI, and GPR concentration in Barcelona Clinic Liver Cancer staging

Factor	BCLC(0+A)	BCLC(B)	BCLC(C)	Z	*p*-value
AFP	21.27 (4.30–253.46)	35.82 (7.18–300.58)	106.12 (10.10–1545.94)	4.061	0.131
sAXL	32.39 (20.72–34.55)	32.61 (28.10–37.47)	34.08 (30.02–37.16)	2.809	0.246
DCP	31.29 (3.09–54.51)	38.74 (5.99–69.62)	46.11 (5.40–274.10)	2.220	0.340
APRI	0.81 (0.45–2.06)	1.19 (0.51–2.38)	1.29 (0.56–2.05)	2.171	0.338
GPR	0.92 (0.38–1.49)	0.90 (0.47–2.07)	1.37 (0.61–2.72)	4.305	0.116

AFP, alpha-fetoprotein; APRI, aminotransferase-to-platelet ratio index; DCP, des-γ-cboxy prothrombin; GPR, gamma-glutamyl transpeptidase-to-platelet ratio; sAXL, soluble AXL.

Diagnostic performance of APRI and GPR serum markers in the detection of HCC

		AFP	sAxl	DCP	APRI	GPR
AFP	Correlation Coefficient	1.00	0.51**	0.49**	0.53**	0.52**
	Sig. (2-tailed)	.	< 0.01	< 0.01	< 0.01	< 0.01
sAxl	Correlation Coefficient	0.51**	1.00	0.47**	0.66**	0.57**
	Sig. (2-tailed)	< 0.01	.	< 0.01	< 0.01	< 0.01
DCP	Correlation Coefficient	0.49**	0.47**	1.00	0.45**	0.44**
	Sig. (2-tailed)	< 0.01	< 0.01	.	< 0.01	< 0.01
APRI	Correlation Coefficient	0.53**	0.66**	0.45**	1.00	0.81**
	Sig. (2-tailed)	< 0.01	< 0.01	< 0.01	.	< 0.01
GPR	Correlation Coefficient	0.52**	0.57**	0.44**	0.81**	1.00
	Sig. (2-tailed)	< 0.01	< 0.01	< 0.01	< 0.01	.

**. Correlation is significant at the 0.01 level (2-tailed).

Abbreviations AFP, alpha-fetoprotein; APRI, aminotransferase-to-platelets ratio index; DCP, Des-γ-cboxy prothrombin; GPR, Gamma-glutamyl transpeptidase-to-platelet ratio; sAxl, soluble Axl.

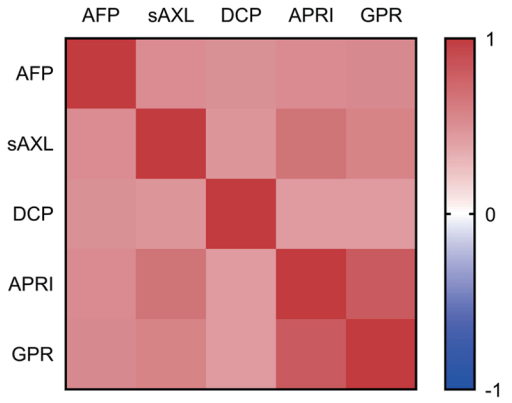

Fig. 2. Correlation analysis of test indicators. AFP, alpha-fetoprotein; APRI, aminotransferase-to-platelet ratio index; DCP, des-γ-carboxy prothrombin; GPR, gamma-glutamyl transpeptidase-to-platelet ratio; sAXL, soluble AXL; red represents positive correlation; blue represents negative correlation.

diagnosing HCC in the Chinese population. Many studies have shown that reasonable multimarker combinations have better sensitivity and specificity than single markers.[22,23] In this study, univariate and multivariate analysis showed that age, sex, AFP, sAXL, DCP, APRI, and GPR were independent risk factors for HCC progression. We also saw significantly higher serum sAXL, DCP, ARRI, and GPR levels in the HCC group than the LC, VH, and HC groups. Those markers may be useful for predicting HCC progression. Also, regular screening for markers or indicators can aid early HCC detection and improve survival rates. Appropriate biomarkers are important for the early screening, diagnosis, treatment, evaluation, and prognosis of liver cancer.[24] The biomarkers that are used are mainly blood-, histochemical-, and drug resistance-related biomarkers. Recently, circulating biomarkers have been extensively studied. Although there are still limitations to be overcome for their widespread clinical application to become possible, basic research regarding those biomarkers is relatively mature. For example, it has been confirmed that CD4 + CD25 + Foxp3 + regulatory T cells have a key role in the immune microenvironment of HCC and have some prognostic significance.[25] In this study, the diagnostic significance of widely used blood biomarkers, AFP, sAXL, and DCP, and two noninvasive indicators, APRI and GPR, were investigated. We used an AFP cutoff value of 10.31 ng/mL for the diagnosis of HCC, with a sensitivity of 73.45% and a specificity of 84.50%. Compared with AFP, sAXL, APRI, and GPR had higher sensitivities at their respective critical values, while DCP showed a higher specificity. We speculated that combining multiple biomarkers might further improve diagnostic performance, and we were not disappointed. Based on our results, it was easy to confirm that the combined use of AFP, DCP, and sAXL significantly improved diagnostic performance. Even though no significant differences in diagnostic value were seen following the inclusion of APRI and GPR, their inclusion still played a certain role in our NRI. The results reported here, which indicated an increase in the overall AUC for HCC detection following the inclusion of APRI and GPR, is supported by published evidence. Specifically, a meta-analysis of whether combined testing of serum markers is effective for improving the clinical value of biomarkers in HCC decision making showed a higher diagnostic potential for combined testing than a single randomized double combination and that combined testing of AFP, AFP-L3, DCP is of clinical importance in HCC decision making.[26] The results of a large-scale multicenter analysis also suggested that combining AFP and sAXL shows high potential as an accurate surveillance marker in patients at high risk for HCC.[27] Similarly, other studies have established a new simple score for the diagnosis of HCC, i.e. the Hepatocellular Carcinoma Multidisciplinary Clinic-Cairo University (HMC-CU) score.[28] Whether the score applies in the Chinese population needs to be further verified. Also, DCP and sAXL

Table 3. Univariate and multivariate analyses of risk factors associated with HCC

Parameter	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Age	1.031 (1.013–1.050)	<0.05	1.049 (1.008–1.092)	<0.05
Sex (male/female)	2.015 (1.384–2.934)	<0.05	3.428 (1.471–7.989)	<0.05
AFP	15.532 (9.642–25.020)	<0.05	4.480 (1.701–11.802)	<0.05
sAXL	6.356 (4.199–9.619)	<0.05	2.857(1.157–7.055)	<0.05
DCP	37.488 (13.306–105.622)	<0.05	16.172 (3.425–76.359)	<0.05
GPR	1.765 (1.367–2.280)	<0.05	3.373(1.129–10.076)	<0.05
APRI	6.905 (4.099–11.630)	<0.05	2.830 (1.019–7.859)	<0.05

AFP, alpha-fetoprotein; APRI, aminotransferase-to-platelet ratio index; CI, confidence interval; DCP, des-γ-cboxy prothrombin; GPR, gamma-glutamyl transpeptidase-to-platelet ratio; HCC, hepatocellular carcinoma; OR, odds ratio; sAXL, soluble AXL.

Table 4. Combined diagnostic performance of APRI, GPR and serum markers in the detection of HCC

Marker	AUC (95%CI)	Sensitivity (%)	Specificity (%)	Youden's index (%)	Cutoff value
AFP	0.825 (0.786–0.859)	73.45	84.50	57.94	10.31
sAXL	0.752 (0.711–0.791)	74.21	72.66	46.87	30.02
DCP	0.738 (0.686–0.786)	53.68	99.22	52.90	19.60
APRI	0.683 (0.636–0.727)	88.07	50.42	38.48	0.38
GPR	0.730 (0.685–0.772)	79.10	60.00	39.10	0.46
AFP+sAXL	0.832 (0.793–0.866)	77.40	78.49	55.89	–
AFP+DCP	0.830 (0.783–0.870)	71.75	94.53	66.28	–
AFP+APRI	0.819 (0.778–0.855)	71.26	86.03	57.29	–
AFP+GPR	0.835 (0.795–0.870)	73.71	81.22	54.94	–
sAXL+DCP	0.882 (0.842–0.915)	82.11	84.37	66.48	–
DCP+APRI	0.822 (0.773–0.864)	69.32	85.59	54.91	–
DCP+GPR	0.823 (0.775–0.865)	64.41	94.07	58.47	–
AFP+sAXL+DCP	0.904 (0.865–0.935)	80.23	90.62	70.85	–
AFP+sAXL+APRI	0.838 (0.798–0.873)	72.41	86.67	59.08	–
AFP+sAXL+GPR	0.832 (0.792–0.867)	65.71	89.78	55.49	–
AFP+DCP+APRI	0.851 (0.805–0.890)	72.99	93.22	66.21	–
AFP+DCP+GPR	0.854 (0.808–0.892)	73.71	94.07	67.78	–
sAXL+DCP+APRI	0.890 (0.849–0.923)	83.52	85.59	69.12	–
sAXL+DCP+GPR	0.890 (0.849–0.924)	84.18	83.90	68.08	–
DCP+APRI+GPR	0.837 (0.790–0.878)	65.34	91.53	56.87	–
AFP+sAXL+DCP+APRI	0.910 (0.871–0.940)	75.86	95.76	71.62	–
AFP+sAXL+DCP+GPR	0.909 (0.870–0.939)	77.71	93.22	70.93	–
AFP+sAXL+APRI+GPR	0.838 (0.799–0.873)	70.11	87.11	57.23	–
AFP+DCP+APRI+GPR	0.853 (0.807–0.891)	73.56	94.92	68.48	–
sAXL+DCP+APRI+GPR	0.891 (0.849–0.924)	85.23	82.20	67.43	–
AFP+sAXL+DCP+APRI+GPR	0.911 (0.872–0.941)	83.91	88.14	72.04	–

AFP, alpha-fetoprotein; APRI, aminotransferase-to-platelet ratio index; AUC, area under the receiver operation characteristics curve; CI, confidence interval; DCP, des-γ-cboxy prothrombin; GPR, gamma-glutamyl transpeptidase-to-platelet ratio; HCC, hepatocellular carcinoma; sAXL, soluble AXL.

assay results cannot be directly compared extensively owing to the use of different techniques and antibodies; however, the strengths of the study include skill and quality control measures to ensure that study procedures are performed accurately. Also, the results may help clinicians diagnose HCC with greater ease, especially at the early stages.

Further investigation of biomarker-related signaling pathways may help to further clarify the mechanism of HCC occurrence and development,[29] to the end of providing a reliable theoretical basis for clinical intervention and treatment. In our initial analysis, we saw positive correlations between AFP, sAXL, DCP, APRI, and GPR, with all the correlation coefficients greater than 0.44. In this study, we further compared the combined diagnostic efficacy of multiple biomarkers for HCC with those of a single biomarker. Thus, we saw that the multimarker groups that showed significantly higher diagnostic values compared with the single biomarkers all included the three markers, AFP, sAXL, and DCP.

Previous studies have shown that AFP promotes HCC proliferation and progression and that there exists an interaction between AFP and retinoic acid receptors.[30] It has also been reported that retinoic acid receptors and retinoid X receptors significantly inhibit the secretion and expression of hepatocyte growth factor (HGF).[31] Notably, HGF is a cellular mesenchymal-epithelial transition factor (c-Met) ligand that can interact with and lead to c-Met phosphorylation, resulting in many effects, such as cell proliferation and invasion. Numerous studies have suggested that c-Met is a target receptor for DCP and that DCP-positive HCC is often accompanied by high levels of phosphorylated c-Met and a high activation rate of its downstream signaling pathways after DCP binding to c-Met.[32] Also, c-Met has been shown to interact functionally with AXL both *in vitro* and *in vivo*, possibly via signaling. Further, drug resistance can occur in patients with tumor recurrence through kinase interactions,[33] and reportedly endogenous and exogenous AXL overexpression induces tumorigenesis, with GAS6/AXL signaling reported to contribute to tumor cell survival. The serum levels of both GAS6 and sAXL are positively correlated with increased tumor staging, and the level of GAS6 is even higher than that of sAXL. It has also been reported that the binding of GAS6 to AXL receptors activates cancer progression, and that AXL expression is up-

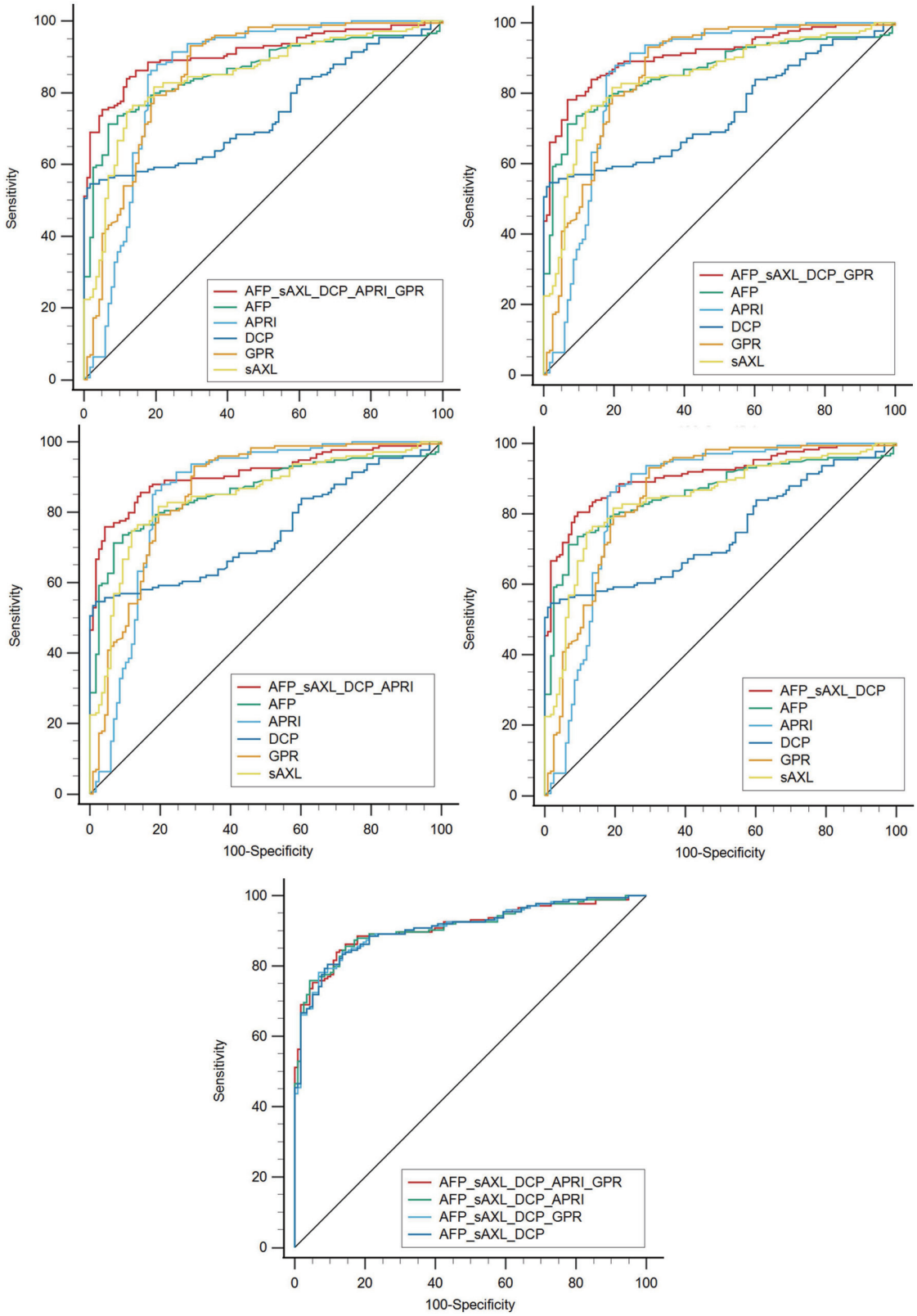

Fig. 3. Pairwise comparison of multiple marker combinations and individual markers to assess their diagnostic performance in HCC using ROC curves. AFP, alpha-fetoprotein; APRI, aminotransferase-to-platelet ratio index; DCP, des-γ-carboxy prothrombin; GPR, gamma-glutamyl transpeptidase-to-platelet ratio; sAXL, soluble AXL.

Table 5. Assessment of diagnostic values in HCC

Group		Standard Error	z statistic	*p*-(AUC)	NRI (%)
AFP+sAXL+DCP+APRI+GPR vs.	AFP	0.0197	2.586	0.0097	37
	sAXL	0.0169	3.796	0.0001	27.6
	DCP	0.0236	7.029	<0.05	20.7
	APRI	0.0265	2.239	0.0251	71.1
	GPR	0.0238	2.176	0.0296	67
AFP+sAXL+DCP+APRI vs.	AFP	0.0196	2.542	0.0110	42.9
	sAXL	0.0163	3.868	0.0001	62
	DCP	0.0236	6.973	<0.05	19.5
	APRI	0.0265	2.203	0.0276	71.2
	GPR	0.0245	2.071	0.0384	69.8
AFP+sAXL+DCP+GPR vs.	AFP	0.0194	2.530	0.0114	42.3
	sAXL	0.0150	4.142	<0.05	51
	DCP	0.0233	7.041	<0.05	19.8
	APRI	0.0243	2.365	0.0180	71.8
	GPR	0.0232	2.151	0.0315	70.4
AFP+sAXL+DCP vs.	AFP	0.0193	2.531	0.0114	36.6
	sAXL	0.0153	4.064	<0.05	34.5
	DCP	0.0230	7.110	<0.05	20.1
	APRI	0.0243	2.360	0.0186	70.2
	GPR	0.0230	2.158	0.0309	67
AFP+sAXL+DCP+APRI+GPR vs. AFP+sAXL+DCP+APRI		0.0022	0.459	0.6460	0.3
AFP+sAXL+DCP+APRI+GPR vs. AFP+sAXL+DCP+GPR		0.0043	0.435	0.6636	1.7
AFP+sAXL+DCP+APRI+GPR vs. AFP+sAXL+DCP		0.0041	0.503	0.6153	2.6
AFP+sAXL+DCP+APRI vs. AFP+sAXL+DCP+GPR		0.0033	0.248	0.8044	3.4
AFP+sAXL+DCP+APRI vs. AFP+sAXL+DCP		0.0034	0.301	0.7635	5.2
AFP+sAXL+DCP+GPR vs. AFP+sAXL+DCP		0.0008	0.229	0.8185	5.2

AFP, alpha-fetoprotein; APRI, aminotransferase-to-platelet ratio index; AUC, area under the receiver operation characteristics curve; DCP, des-γ-cboxy prothrombin; GPR, gamma-glutamyl transpeptidase-to-platelet ratio; HCC, hepatocellular carcinoma; NRI, net reclassification improvement; sAXL, soluble AXL.

regulated in HCC. Elevated sAXL levels have also been seen in cirrhosis and early to advanced stages of HCC.[34] Considering previous findings and the study results, there may be a novel mechanism involving the AFP-HGF/c-MET-AXL/GAS6 axis by which the progression of HCC is promoted; however, further *in vitro* and *ex vivo* experiments are needed to verify this speculation.

In simple terms, c-Met is the target receptor of DCP, while sAXL is the soluble part of AXL, and our results in this study revealed that DCP, sAXL, and their combined detection showed good performance regarding HCC diagnosis (c-Met and AXL are tyrosine kinases). So we believe that targeted drugs related to tyrosine kinase inhibitors are worthy of attention for the diagnosis and treatment of HCC. Further, the efficacy and safety of drugs targeting c-MET and AXL, such as cabozantinib, in patients with advanced HCC have also been reported recently. As expected, an earlier study showed that cabozantinib improves the overall and progression-free survival of treated patients with HCC.[35] Further, in a Phase Ib study, cabozantinib treatment transformed locally advanced HCC into a resectable tumor by enhancing antitumor immunity.[36] Therefore, tyrosine kinase receptor inhibitors, such as c-Met/AXL targets may improve the status of patients with HCC; however, a large number of clinical trials are still needed for validation.

This study had limitations. First, we did not obtain complete *in vivo* and *in vitro* experimental results to verify the potential mechanisms of the interactions between biomarkers. Second, the patient population was small given our strict exclusion criteria and the univariate and multivariate analyses performed.

Conclusions

The results indicated that AFP, sAXL, DCP, APRI, and GPR were independent risk factors for HCC. The diagnostic performance of AFP with sAXL, DCP, APRI, and GPR was superior to that of the individual biomarkers in relation to HCC diagnosis. Further, although no significant differences in diagnostic performance were seen on comparing the diagnostic values based on AFP, sAXL, and DCP with or without APRI and GPR, including APRI and GPR resulted in an increased NRI. Also, the correlation between the expression of the biomarkers suggested there might be pathways, such as the AFP-HGF/c-

MET-AXL/GAS6 axis, that promote HCC progression; however, further validation studies are needed.

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Conflict of interest

The authors have no conflicts of interest related to this publication.

Author contributions

Data organization and drafting of the manuscript (CXF), data collection (JL), data analysis and interpretation (ZDC), sample collection and processing (YPC, HLZ, BCS, LX, YZ, MZ), study concept and design, administrative, technical, or material support (HXP, EYY).

Ethical statement

This study was approved by the Ethics Committee of the Affiliated Hospital of Yanbian University, Yanji, China. The study was part of a project named, "Case-control study on factors influencing disease progression of hepatitis C and hepatitis B: National Twelfth 5-Year Plan Science and Technology Major Project" (approval number 20130064). All the participants signed a written informed consent form, and the study conducted in compliance with the principles of the Declaration of Helsinki and the Ethical Review of Biomedical Research Involving Human Beings.

Data sharing statement

No additional data are available.

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