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ORIGINAL RESEARCH

The Diagnostic Performance of AFP, AFP-L3, DCP, CA199, and Their Combination for Primary Liver Cancer

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Purpose: The prevalence of primary liver cancer (PLC) is rising, yet strategies for its early diagnosis remain inadequate. This study aims to identify novel biomarkers to improve the diagnostic ability of PLC.

Patients and Methods: This study included 94 patients with PLC, 128 patients with benign liver disease (BLD), and 79 normal controls (NC) were included. Among the PLC group, there were 39 patients with hepatocellular carcinoma (HCC), 14 patients with intrahepatic cholangiocarcinoma (ICC), 4 patients with combined hepatocellular-cholangiocarcinoma (CHC) and 37 patients with imaging-diagnosed HCC, respectively. Serum biomarkers and other laboratory parameters were collected and analyzed. Diagnostic values of individual and combined biomarkers for PLC were assessed using receiver operating characteristic (ROC) curve analysis. Univariate and multivariate logistic regression identified predictors of PLC, and a nomogram model was developed based on the independent predictors.

Results: AFP and DCP levels were significantly higher in the HCC patients compared to those with the BLD. AFP-L3 and CA199 levels were markedly elevated in patients with HCC, ICC, and CHC compared with the other groups. Combining AFP, AFP-L3, DCP, and CA199 increased the AUC to 0.8492 for the PLC group versus the BLD group. Multivariate logistic regression analysis identified sex, AFP-L3, DCP, and CA199 as independent predictors of PLC, and a reliable nomogram model was developed based on these predictors.

Conclusion: The combined use of AFP, AFP-L3, DCP, and CA199 significantly enhanced the diagnostic performance of PLC compared with existing models like GALAD (gender, age, AFP, AFP-L3, and DCP), and ASAP (age, sex, AFP, DCP), as well as individual biomarkers.

Keywords: serum biomarkers, ROC curve, nomogram, early diagnosis

Introduction

Primary liver cancer (PLC) is the most common malignancy of the digestive system and the fourth leading cause of cancer-related mortality worldwide.[1] PLC encompasses three main subtypes: hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC), and combined hepatocellular-cholangiocarcinoma (CHC). Among these, HCC accounts for 80–90% of cases, while ICC represents 10%-15%.[2] CHC is the rarest subtype, accounting for 1–4.7% of PLC.[3] Typically, PLC is diagnosed at advanced stages, resulting in limited treatment options and a poor 5-year survival rate.[4] Early detection and timely treatment are critical for improving survival outcomes.[5] Current clinical guidelines recommend biannual surveillance for HCC in high-risk patients using abdominal ultrasonography (US) and serum alpha-fetoprotein (AFP) testing.[6,7] The efficiency of the US in detecting early-stage HCC is limited by the operator's expertise and patient-specific characteristics. The combination of US and AFP has been reported to have a sensitivity of only 63% for early-stage HCC detection.[8] Furthermore, AFP alone demonstrates limited sensitivity, ranging from 40%-60% and approximately 40% of HCC patients, particularly those in early stages, have normal AFP levels.[9,10] These findings highlight the need for more reliable serum biomarkers for early liver cancer detection.

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Des-gamma-carboxy prothrombin (DCP) and lens culinaris agglutinin-reactive AFP (AFP-L3), have been utilized for HCC screening.[11–14] The combination of DCP and AFP-L3 with AFP has shown promise in improving sensitivity for early HCC detection.[15–18] Lim et al demonstrated that combining AFP, DCP, and AFP-L3 improved the sensitivity and specificity to 87.0% and 60.1%, respectively, in diagnosing HCC among cirrhotic patients.[19] Other liver function biomarkers, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), have also shown potential in aiding HCC diagnosis and prognosis.[20,21] Novel diagnostic algorithms, such as GALAD (gender, age, AFP, AFP-L3, and DCP), have been developed to further enhance HCC detection.[22,23] The GALAD score significantly improves diagnostic performance, with reported sensitivity and specificity of approximately 85.6% and 93.3%, respectively, outperforming individual biomarkers.[24,25] Similarly, the ASAP (age, sex, AFP, and DCP) score, derived from Chinese cohort of HBV-infected patients, demonstrated sensitivity and specificity of approximately 76.1% and 90.4%, respectively, for early HCC detection.[26] Nevertheless, the performance of GALAD and ASAP diminishes in AFP-negative HCC case, emphasizing the need for optimized biomarker panels.[27]

ICC, the second most prevalent subtype of PLC, has a dismal 5-year overall survival (OS) rate of approximately 9%.[28] Although ICC diagnosis primarily relies on imaging and tissue biopsy, serum biomarkers such as carbohydrate antigen 50 (CA50), carbohydrate antigen 242 (CA242), carbohydrate antigen 199 (CA199), and carcinoembryonic antigen (CEA) are also clinically significant.[29] Elevated CA50 serum levels have been observed in ICC patients, distinguishing them from non-ICC controls. Furthermore, higher CA50 levels are associated with poorer clinical outcomes and shorter survival in ICC patients.[30] Similarly, serum CA242 levels are significantly lower in ICC patients after treatment, indicating its potential as a biomarker for treatment monitoring.[31] ICC patients often exhibit elevated serum CA199 levels, which demonstrate 72% sensitivity and 84% specificity for ICC diagnosis.[32] Additionally, elevated serum levels of CA199 and CEA are frequently observed in patients with locally advanced or metastatic ICC.[33–35] Considering the pathological characteristics of CHC patients are similar to those of HCC and ICC, the serum levels of AFP, DCP, CEA, and CA199 are elevated in CHC patients.[28] However, these biomarkers have not been considered effective enough for clinical application as indicators for ICC and CHC diagnosis.

To improve the early diagnosis of PLC, it is necessary to combine the current tumor biomarkers to enhance the diagnosis accuracy for PLC. This study retrospectively evaluated the diagnostic performance of a biomarker panel comprising AFP, AFP-L3, DCP, and CA199 in combination for diagnosing PLC.

Materials and Methods

Ethics Statement

The study was approved by the Ethics Committee of Huadong Hospital (project number 20160089) and conducted in accordance with the Declaration of Helsinki. All participants were over 18 years old and voluntarily provided written informed consent.

Patient Subjects

This retrospective study included 94 patients diagnosed with primary liver cancer (PLC), 128 patients diagnosed with benign liver disease (BLD), and 79 normal controls (NC). The PLC group comprised 39 patients with hepatocellular carcinoma (HCC), 14 patients with intrahepatic cholangiocarcinoma (ICC), 4 patients with combined hepatocellular-cholangiocarcinoma (CHC), and 37 diagnosed with HCC based on imaging. All participants were recruited from Huadong Hospital between June 2020 and June 2024. PLC diagnoses were confirmed through histopathological examination or, when unavailable, radiological evidence following the Liver Imaging Reporting and Data System (LI-RADS). Data were collected from newly diagnosed, untreated PLC patients. Exclusion criteria were as follows: (1) PLC recurrence; (2) non-PLC liver metastases; (3) presence of other tumors; and (4) treatment with warfarin or vitamin K, which could affect DCP serum levels. Tumor staging was determined according to the eighth edition of the Union for International Cancer Control (UICC) TNM classification and Barcelona Clinical Liver Cancer (BCLC) staging system.[36] The BLD group included patients diagnosed with chronic liver disease (CLD), liver fibrosis (LF) or cirrhosis (LC).

Biomarkers Determination

Tumor biomarkers tests: AFP, CEA, and CA199 levels were measured using the Roche Cobas E601 electrochemical immunoluminescence analyzer and its corresponding reagent kit (Roche Diagnostics, Geneva, Switzerland). AFP-L3 levels were measured by the μTASWako i30 auto-analyzer and its reagent kit (Wako Pure Chemical Corporation, Osaka, Japan). DCP levels were measured using the ARCHITECT immunoassay system and its supporting reagent kit (Abbott Diagnostics, Chicago, IL, USA). CA50 and CA242 levels were analyzed using the Smart 6500 analyzer and its supporting reagent kit (Tellgen Corporation, Shanghai, China).

Liver function tests: ALT, AST, ALP, and gamma-glutamyl transferase (GGT) were measured using the Roche Cobas 8000 analyzer and its supporting reagent kit (Roche Diagnostics, Geneva, Switzerland). Total bilirubin (TB), direct bilirubin (DB), and albumin (ALB) were also measured using the Roche Cobas 8000 analyzer and its supporting reagent kit (Roche Diagnostics, Geneva, Switzerland). Prothrombin time (PT) was assessed with the ACL Top 700 automatic coagulation analyzer (Instrumentation Laboratory Company, Barcelona, Spanish), while Platelet (PLT) count was measured using the Mindray BC-6800 hematological analyzer (Mindray Corporation, Shenzhen, China). Hepatitis B surface antigen (HBsAg) and anti-HCV antibodies were evaluated with the Architect I2000 (Abbott Diagnostics, Chicago, IL, USA).

Calculation of the GALAD and ASAP Score

The GALAD score was calculated based on gender, age, AFP, AFP-L3, and DCP levels using the following formula: GALAD score = −10.08 + 1.67 × gender (1 for males, 0 for females) + 0.09 × age + 0.04 × AFP-L3% + 2.34 × $\log_{10}$AFP + 1.33 × $\log_{10}$DCP.[18] The ASAP score was calculated using the following equation: ASAP score = −7.58 + 0.05 × age - 0.58 × sex (1 for females, 0 for males) + 0.42 × Ln (AFP) + 1.11 × Ln (DCP).[26]

Statistical Analysis

Statistical analyses were performed using R (https://www.r-project.org), Prism (GraphPad, La Jolla, CA), and SPSS 28.0.1.0 (SPSS Inc., an IBM Company, and Chicago, IL, USA). Categorical variables were presented as frequencies and percentages, while continuous variables were reported as means (standard deviation) or medians (interquartile range). Continuous variables were compared using analysis of variance (ANOVA), Student's *t*-test or the Mann–Whitney *U*-test, while categorical variables were compared using chi-square tests. Receiver operating characteristic (ROC) curves were used to determine the area under the curve (AUC) for individual biomarkers (AFP, AFP-L3, DCP, CA199), the GALAD score, and the ASAP score, and combinations of these four serum biomarkers in predicting PLC. Youden's index was used to determine optimal cutoff values for sensitivity and specificity comparisons. Positive predictive value (PPV) and negative predictive value (NPV) were calculated based on the following clinical thresholds: AFP ≥ 20 ng/mL, AFP-L3 > 10%, DCP > 40 mAU/mL, and CA199 > 34 U/mL. Univariate and multivariate logistic regression analyses were conducted to identify the independent prognostic factors. Based on these factors, a predictive nomogram model was developed. A two-tailed value of $p<0.05$ was considered statistically significant.

Results

Characteristics of the Enrolled Population

A total of 301 subjects were included in this retrospective study. The general clinical information of subjects in the NC, BLD, and PLC groups is summarized in Table 1. The mean age of participants in the BLD and PLC groups was higher than that in the NC group (62.39, 63.94, and 57.44 years, respectively). The PLC group also exhibited a higher prevalence of males (77.66%). Serum concentrations of tumor biomarkers, including AFP, DCP, AFP-L3, CEA, CA199, and CA50, were significantly elevated in PLC patients compared to those in the BLD group or normal controls ($p < 0.001$). Similarly, the PLC group demonstrated significantly higher levels of liver function biomarkers, such as ALT, AST, TB, DB, ALP, GGT, and PT ($p < 0.001$), while ALB and PLT levels were significantly lower compared to other groups ($p< 0.001$).

Table 1 Baseline Characteristics of the Enrolled Population

	NC Group (n=79)	BLD Group (n=128)	PLC Group (n=94)	*p* value
Age, years *	57.44±13.32 (31–85)	62.39±13.82 (20–96)	63.94±12.22 (29–87)	0.004
Sex Male Female	 32 (40.5%) 47 (59.49%)	 55 (42.97%) 73 (57.03%)	 73 (77.66%) 21 (22.34%)	0.001
AFP (ng/mL)	2.9 (2.2–4.5)	7.6 (2.95–23.43)	6.8 (2.9–641.85)	0.001
DCP (mAU/mL)	21.73 (17.64–28.56)	22.77 (14.57–37.04)	81.7 (26.35–1512.21)	0.001
AFP-L3 (%)	0.5 (0.5–0.5)	2.91 (0.5–0.5)	5 (0.5–35.8)	0.001
CEA (ng/mL)	1.7 (1.3–2.7)	2.55 (1.73–3.78)	2.7 (1.8–3.7)	0.001
CA199 (U/mL)	7.4 (4.3–12.4)	11.1 (4–28.8)	21.35 (11.18–47.18)	0.001
CA50 (U/mL)	2.69 (1–4.27)	7.47 (3.4–16.53)	7.77 (3.69–14.4)	0.001
CA242 (U/mL)	3.75 (2.25–6.71)	4.14 (2.28–7.3)	4.05 (2.65–7.81)	0.533
ALT (U/L)	17.6 (13.3–22)	24.85 (14.45–68.65)	28.25 (18.17–51.05)	0.001
AST (U/L)	19 (15.9–23.5)	26.45 (9.58–57.3)	33.8 (25–55.65)	0.001
TB (μmol/L)	11.1 (8.8–14)	18.5 (12.03–46.25)	15 (9.83–23.48)	0.001
DB (μmol/L)	4.2 (3.4–5)	13.2 (5.35–33.58)	7.2 (4.55–11.9)	0.001
ALB (g/L)	46.1 (43.9–48)	37.85 (31.83–43.8)	39.3 (34.8–42.2)	0.001
ALP (U/L)	75.5 (64.3–89.2)	89 (47.28–140.55)	103.15 (78.78–182)	0.001
GGT (U/L)	24 (14.6–32.6)	73 (38.43–138.38)	66.45 (35.5–134.7)	0.001
PT (s)	11.5 (11.1–12.3)	12.1 (11.3–13.3)	12.6 (11.7–13.45)	0.001
PLT (10^9/L)	221 (180–249)	180 (133–229)	146 (88–206)	0.001

Notes: *As mean and standard deviation for age, and as median and interquartile ranges (IQR) for other continuous variables. Categorical variables were presented as frequencies and percentages. Analysis of variance (ANOVA), Mann–Whitney *U*-test, and chi-square test were applied where appropriate.

Demographic and Clinicopathologic Characteristics Between AFP-Negative and AFP-Positive PLC Patients

To explore the differences between AFP-positive and AFP-negative PLC patients, we analyzed their clinicopathological data (Table 2). There were no significant differences in age ($p = 0.982$), sex distribution ($p = 0.368$), or HBsAg status ($p = 0.894$), with HBsAg positivity observed in 75% in the AFP-negative group and 76.19% in the AFP-positive patients. Tumor biomarker analysis revealed that AFP-L3 levels were significantly higher in the AFP-positive group (27.46%) than in the AFP-negative group (0.5%) ($p = 0.001$). Similarly, DCP levels were significantly higher in AFP-positive patients (315.2 mAU/mL) compared to AFP-negative patients (33.91 mAU/mL) ($p = 0.002$). No significant differences were observed in other biomarkers, such as CEA ($p = 0.369$) and CA242 ($p = 0.422$). Among liver function biomarkers, AST levels were significantly elevated in AFP-positive PLC patients ($p = 0.009$). However, histological grade showed no significant differences, with most cases in both groups classified as moderately differentiated tumors (76.92% in AFP-negative and 66.67% in AFP-positive, $p = 0.358$). Other clinicopathological factors, including microvascular invasion (MVI) status ($p = 0.104$), tumor count ($p = 0.519$), and BCLC stage ($p = 0.191$), also showed no significant differences.

Table 2 Demographic and Clinicopathologic Characteristics of AFP-Negative and AFP-Positive PLC Patients

	AFP⁻(<20ug/mL)	**AFP⁺ (≥ 20ug/mL)**	***p* value**
Age, years*	63.96±13.65 (29–87)	63.9±10.34 (44–84)	0.982
Sex Male Female	 39 (75%) 13 (25%)	 34 (80.95%) 8 (19.05%)	0.368
AFP (ng/mL)	3.05 (2.1–4.48)	804.2 (142.8–3181.5)	0.001
AFP-L3 (%)	0.5 (0.5–6.43)	27.4 (6.45–73.9)	0.001
DCP (mAU/mL)	33.91 (20.37–318.62)	315.2 (58.45–2620.12)	0.002
CEA (ng/mL)	2.75 (1.83–3.76)	2.4 (1.75–3.5)	0.369
CA199 (U/mL)	18 (9.13–54.45)	23.8 (13.58–40.53)	0.566
CA242 (U/mL)	3.95 (2.42–7.51)	4.27 (2.9–8.1)	0.422
CA50 (U/mL)	6.79 (3.63–13.91)	9.36 (4.21–14.43)	0.376
HBsAg Negative Positive	 13 (25%) 39 (75%)	 10 (23.81%) 32 (76.19%)	0.894
HCV-Ab Negative Positive	 52 (100%) 0 (0%)	 38 (90.48%) 4 (9.52%)	0.117
TB (μmol/L)	14.55 (9.4–20.86)	16.45 (10.88–31.45)	0.182
DB (μmol/L)	6.55 (4.5–9.98)	8 (4.8–14.85)	0.209
ALT (U/L)	24.3 (17.38–43.93)	37.05 (23.2–55.63)	0.089
AST (U/L)	30 (20.25–44.65)	45.55 (28.63–63.28)	0.009
ALP (U/L)	98.55 (81.1–183.125)	108.4 (74.58–182.83)	0.811
GGT (U/L)	57.4 (29.35–111.95)	78 (38.78–148.38)	0.205
ALB (g/L)	39.75 (35–42.48)	38.5 (34.63–42)	0.465
PLT (10^9/L)	163 (90–210)	133 (86–191)	0.139
PT(s)	12.1 (11.7–13.2)	12.8 (11.75–14)	0.179
Child-Pugh grade A+B C	 49 (94.23%) 3 (5.77%)	 40 (95.24%) 2 (4.76%)	0.828
Imaging diagnosis	13 (35.14%)	24 (64.86%)	
Tumor type HCC ICC CHC	 25 (64.10%) 12 (30.77%) 2 (5.13%)	 14 (77.78%) 2 (11.11%) 2 (11.11%)	0.234

(*Continued*)

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Table 2 (Continued).

	AFP^-(<20ug/mL)	AFP^+ (≥ 20ug/mL)	*p* value
Histologic grade Poor Mediate Well	 7 (17.95%) 30 (76.92%) 2 (5.13%)	 3 (16.67%) 12 (66.67%) 3 (16.67%)	0.358
Ki-67 <20% ≥20%	 11 (28.20%) 28 (71.79%)	 7 (38.89%) 11 (61.11%)	0.542
MVI M0 M1+M2	 19 (48.72%) 20 (51.28%)	 4 (22.22%) 14 (77.78%)	0.104
Tumor size(cm) ≤ 5 >5	 27 (51.92%) 25 (48.08%)	 16 (38.10%) 26 (61.90%)	0.181
Number of tumors Solitary Multiple	 27 (51.92%) 25 (48.08%)	 19 (45.24%) 23 (54.76%)	0.519
Clinical TNM stage I+II III+IV	 28 (53.85%) 24 (46.15%)	 16 (38.10%) 26 (61.90%)	0.128
BCLC stage 0-A B-D	 15 (60%) 10 (40%)	 12 (85.71%) 2 (14.29%)	0.191
ECOG PS ≤1 ≥2	 40 (76.92%) 12 (23.08%)	 34 (80.95%) 8 (19.05%)	0.635

Notes: *As mean and standard deviation for age, and as median and interquartile ranges (IQR) for other continuous variables. Categorical variables were presented as frequencies and percentages. Student's *t*-test, Mann–Whitney *U*-test, and chi-square test were applied where appropriate.

In summary, AFP-positive PLC patients exhibited significantly higher levels of AFP-L3, DCP, and AST compared to AFP-negative patients, while other demographic, tumor biomarkers and clinicopathological characteristics were similar.

Evaluation of PLC-Associated Serum Biomarkers in PLC, BLD, and NC Groups

To determine the differences in serum levels of PLC-associated tumor serum biomarkers, data for seven biomarkers were compared across groups (Figure 1). Serum levels of AFP and DCP were significantly higher in the HCC group compared with those in the BLD groups ($p < 0.001$ and $p < 0.001$, respectively) (Figure 1A-B). However, AFP levels were not elevated in the ICC and CHC groups compared with the BLD group (Figure 1A), while DCP serum levels were elevated in the CHC group compared with those in the BLD group ($p < 0.05$) (Figure 1B).

Furthermore, the GALAD and ASAP scores were also significantly higher in the HCC and CHC groups than that in the BLD group ($p < 0.001$ and $p < 0.05$, respectively) (Figure 1H-I). AFP-L3 levels were higher in the HCC, ICC, and CHC groups compared to the BLD group ($p < 0.001$, $p < 0.05$, and $p < 0.01$, respectively) (Figure 1C). Interestingly, the serum levels of CA199 in HCC, ICC, and CHC groups were higher than those in the BLD group ($p < 0.05$, $p < 0.001$, and $p < 0.001$, respectively) (Figure 1D). However, CEA levels were only elevated in the ICC group ($p < 0.001$) (Figure 1E). CA242 levels were elevated in ICC and CHC groups, but not in the HCC group ($p < 0.01$, $p < 0.001$, and $p > 0.05$, respectively) (Figure 1G).

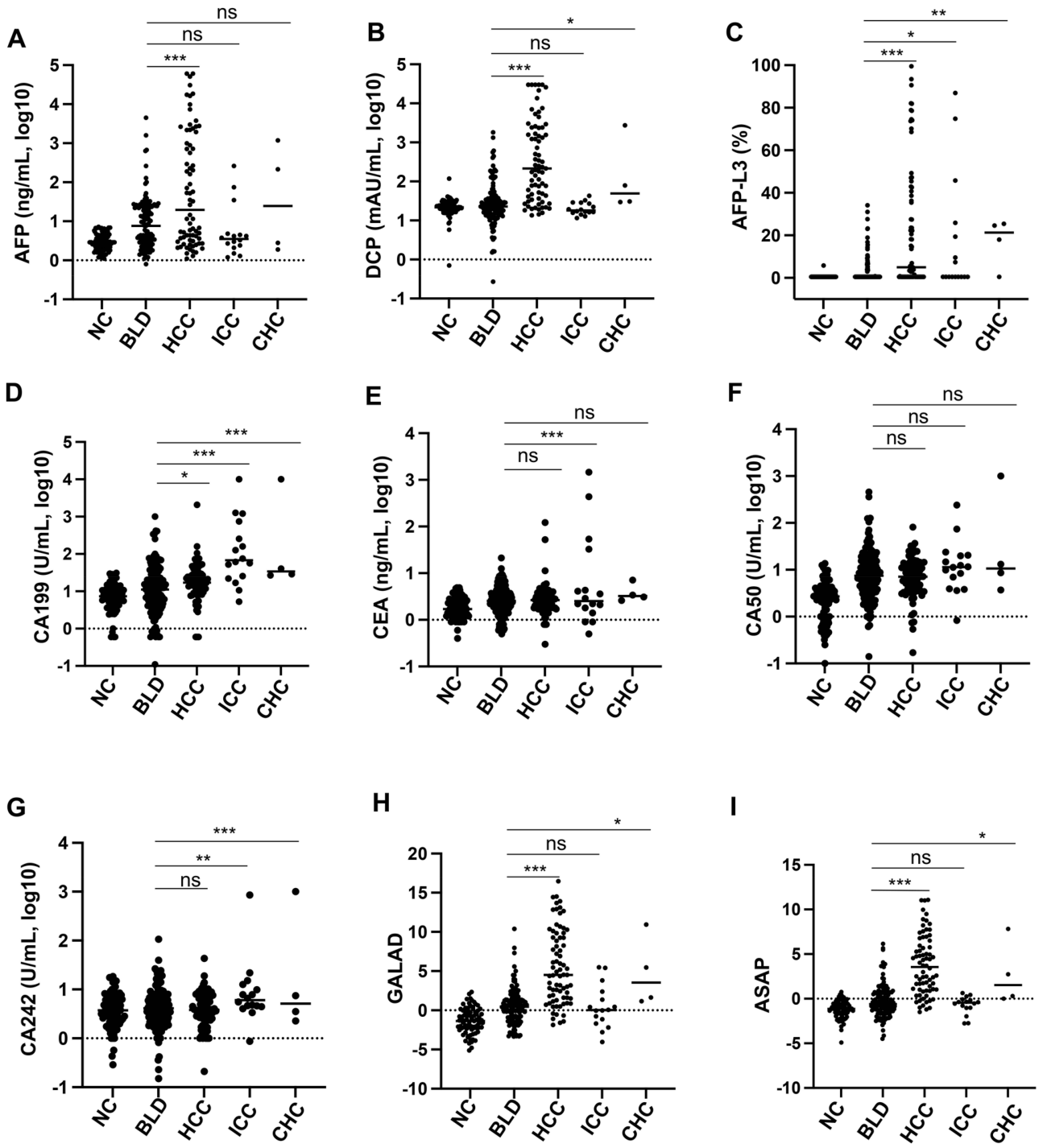

Figure 1 Serum levels of AFP (**A**), DCP (**B**), AFP-L3 (**C**), CA199 (**D**), CEA (**E**), CA50 (**F**), CA242 (**G**), GALAD score (**H**), ASAP (**I**) in NC, BLD, HCC, ICC, and CHC groups. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, ns $p > 0.05$, no significance.

No significant differences in CA50 levels were observed between the HCC, ICC, CHC, and BLD groups (Figure 1F). These findings indicate that AFP, AFP-L3, DCP, and CA199, may serve as individual diagnostic biomarkers for HCC.

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Evaluation of the Diagnostic Values of AFP, AFP-L3, DCP, CA199, and Their Combination for PLC Patients

ROC curve analysis was used to evaluate the diagnostic performance of the four biomarker panels including AFP, AFP-L3, DCP, and CA199, and their combination, as well as the GALAD score and the ASAP scores for differentiating PLC from BLD (Figure 2A and Table 3). The area under the ROC curve (AUC) for AFP, AFP-L3, DCP, and CA199 were 0.5928, 0.7229, 0.764, and 0.6523, respectively. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) are presented in Table 3. CA199 exhibited the highest sensitivity of 0.8723 while AFP showed the highest specificity of 0.9375. Combining AFP, AFP-L3, DCP, and CA199 improved diagnostic performance, increasing the AUC to 0.8492, surpassing both the GALAD score (AUC = 0.7751) and the ASAP score (AUC = 0.7927). The ASAP score exhibited a high PPV (0.82) for the PLC detection compared to BLD. We subsequently validated the diagnostic performance of these biomarkers in the PLC group compared with the NC group (Figure 2B), and the AUC for AFP, AFP-L3, DCP, and CA199 were 0.7267, 0.7938, 0.801, and 0.7912, respectively (Table 4). The combination of AFP, AFP-L3, DCP, and CA199 increased the AUC to 0.9480, which surpassed that of the GALAD score (AUC = 0.9022) and the ASAP score (AUC = 0.9016). The combination of AFP, AFP-L3, DCP, and CA199 exhibited a sensitivity of 0.8404 and a specificity of 0.9873. These findings suggested that combining AFP, AFP-L3, DCP, and CA199 could enhance their diagnostic ability to differentiate patients with PLC from those with BLD or NC.

Nomogram for Predicting PLC Based on Logistic Regression

Univariate and multivariate logistic regression analyses identified sex, AFP-L3, DCP, and CA199, as independent predictors of PLC when compared to BLD (Table 5). In the multivariate logistic regression analysis, male (OR: 3.99, 95% CI 1.92–8.71; $p = 0.001$), increased AFP-L3 (OR: 1.071, 95% CI 1.061–1.32; $p = 0.001$), elevated DCP (OR: 1.001, 95% CI 1–1.002; $p = 0.02$), increased CA199 (OR: 1.001, 95% CI 1–1.005; $p = 0.04$) were independent predictors of PLC. Based on these factors, a nomogram was established for PLC diagnosis (Figure 3A). The nomogram demonstrated strong discriminatory power, with a C-index of 0.878 (Figure 3B).

Discussion

Primary liver cancer (PLC), one of the most common malignant tumors, poses a significant threat to public health. Currently, AFP and US are the most commonly recommended tests for PLC surveillance in cirrhotic patients.[37,38] However, due to the often asymptomatic nature of early-stage PLC, many PLC patients are diagnosed at advanced stages.

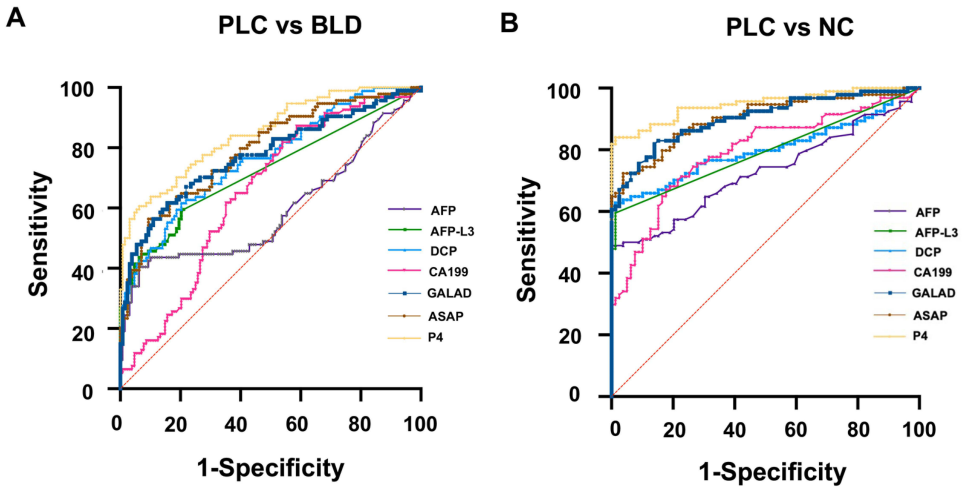

Figure 2 The diagnostic performance of AFP, AFP-L3, DCP, CA199, their combination P4, GALAD, and ASAP for PLC patients. (**A**) ROC curves of AFP, AFP-L3, DCP, CA199, their combination P4, GALAD, and ASAP in distinguishing the PLC patients from the BLD patients. (**B**) ROC curves of AFP, AFP-L3, DCP, CA199, their combination P4, GALAD, and ASAP in distinguishing the PLC patients from the NC group.

Table 3 The ROC Curve Analyses of AFP, AFP-L3, DCP, CA199, and Their Combination P4, GALAD, and ASAP Between PLC and BLD Patients

Panel	*p* value	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC (95% CI)
AFP	0.018	51.95	0.4043	0.9375	0.51	0.63	0.5928 (0.5118–0.6737)
AFP-L3	0.0001	0.65	0.5957	0.7891	0.79	0.69	0.7229 (0.6523–0.7936)
DCP	0.0001	43.02	0.617	0.7969	0.67	0.74	0.764 (0.7009–0.8272)
CA199	0.0001	7.4	0.8723	0.4141	0.49	0.60	0.6523 (0.5804–0.7242)
P4	0.0001	–	0.6064	0.9375	–	–	0.8492 (0.7986–0.8998)
GALAD	0.0001	2.245	0.617	0.8438	0.73	0.75	0.7751 (0.7103–0.8398)
ASAP	0.0001	1.615	0.5638	0.9063	0.82	0.74	0.7927 (0.7326–0.8528)

Table 4 The ROC Curve Analyses of AFP, AFP-L3, DCP, CA199, and Their Combination P4, GALAD, and ASAP Between PLC Patients and NC Group

Panel	*p* value	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC (95% CI)
AFP	0.0001	8	0.4894	1	1	0.6	0.7267 (0.6512–0.8022)
AFP-L3	0.0001	0.65	0.5957	0.9873	1	0.6	0.7938 (0.7261–0.8615)
DCP	0.0001	42.03	0.617	1	0.98	0.69	0.801 (0.7329–0.869)
CA199	0.0001	14.35	0.6702	0.8228	1	0.54	0.7912 (0.7239–0.8585)
P4	0.0001	–	0.8404	0.9873	–	–	0.9480 (0.9159–0.9802)
GALAD	0.0001	0.43	0.8191	0.8608	0.84	0.65	0.9022 (0.8572–0.9473)
ASAP	0.0001	0.25	0.7234	0.962	0.95	0.65	0.9016 (0.8560–0.9471)

Table 5 Univariate and Multivariate Logistic Regression Analysis Between PLC Patients and BLD Patients

Parameters	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Factors Selected				
Sex	4.614(2.536–8.393)	0.001	3.99(1.92–8.71)	0.001
AFP-L3	1.081(1.051–1.121)	0.001	1.071(1.061–1.32)	0.001
DCP	1.002(1.001–1.003)	0.001	1.001(1–1.002)	0.02
CA199	1.002(1–1.003)	0.012	1.001(1–1.005)	0.04

(*Continued*)

Table 5 (Continued).

Parameters	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	***p* value**	**OR (95% CI)**	***p* value**
Factors not Selected				
AFP	1.001(1.00–1.002)	0.006	1(1–1.001)	0.28
PLT	0.994(0.99–0.9978)	0.002	0.996(0.991–1)	0.05
Age	1.009(0.99–1.03)	0.3869		
CEA	1.032(1.002–1.098)	0.179		
CA50	1.001(0.9969–1.005)	0.357		
CA242	1.004(0.9995–1.022)	0.316		
ALT	0.9981(0.995–1)	0.15		
AST	0.9991(0.9965–1.002)	0.49		
TB	0.995(0.9883–0.9996)	0.08		
DB	0.9935(0.985–0.999)	0.07		
ALB	1.007(0.968–1.05)	0.72		
ALP	1(0.9989–1.002)	0.69		
GGT	0.9994(0.9979–1.001)	0.37		
PT	1.023(0.9119–1.147)	0.69		

Early diagnosis is crucial for effective treatment, improved prognosis, and enhanced long-term survival.[39] This underscores the urgent need for efficient, non-invasive prognostic tools to improve PLC detection.

Various biomarkers, such as AFP, AFP-L3, and DCP, have been extensively studied for PLC detection.[25,40] Although AFP is widely used for PLC surveillance, about 30–50% of PLC patients are AFP-negative.[19] In our cohort, 55.4% of PLC patients in our cohort were AFP-negative. Moreover, AFP showed a relatively low PPV of 0.51, indicating a higher rate of false positives, likely due to AFP's non-specific elevation in BLD patients such as chronic hepatitis or cirrhosis. Several studies have shown that DCP and AFP-L3 improve diagnostic accuracy, particularly for AFP-negative HCC, and are potential predictors of early HCC recurrence.[40–43] Consistent with other studies, we found that DCP had the highest NPV (0.74) and AFP-L3 exhibited the highest PPV (0.79) for distinguishing PLC from BLD patients, suggesting fewer false positives and false negatives compared to AFP. However, false negatives in DCP may occur in early-stage PLC cancers, and false positives in AFP-L3 might still occur in BLD patients. Given these limitations, combining complementary biomarkers may enhance diagnostic accuracy. A previous study demonstrated that CA199 in the liver cancer group was significantly higher than that in the benign lesion group, and CA199 in the liver cancer patients, with a combination of AFP, and imaging (eg US or CT) was achieving an AUC of 0.962.[44] In our study, CA199 was significantly elevated in the HCC, ICC, and CHC groups. Intriguingly, the combination of AFP, AFP-L3, DCP, and CA199 achieved superior diagnostic performance, with AUCs of 0.8492 and 0.9480 for discriminating PLC patients from BLD and NC groups, respectively.

As previously mentioned, The GALAD and ASAP scores are well-established tools for estimating HCC risk and have demonstrated improved sensitivity for HCC detection.[26,45,46] Consistent with these reports, we found that GALAD and the ASAP scores outperform individual biomarkers (AFP, AFP-L3, or DCP) in detecting PLC. Moreover, our results demonstrated that the combination of AFP, AFP-L3, DCP, and CA199 surpasses the ASAP and GALAD scores in diagnostic performance. This improved performance may be attributed to the inclusion of CA199, which is elevated not only in HCC but also in ICC and CHC. Differences in the study populations may also contribute to these findings, as GALAD and ASAP studies typically focus on solely on HCC patients, whereas our study included HCC, ICC, and CHC patients, in which CA199 is not only elevated in HCC but also significantly elevated in ICC and CHC patients. We also developed a nomogram model to predict PLC risk based on independent predictors, including sex, AFP-L3, DCP, and CA199. This model highlights the potential of these factors as predictive tools for liver cancer. Notably, the combined biomarker tests are convenient and noninvasive, which may improve surveillance compliance in high-risk populations.

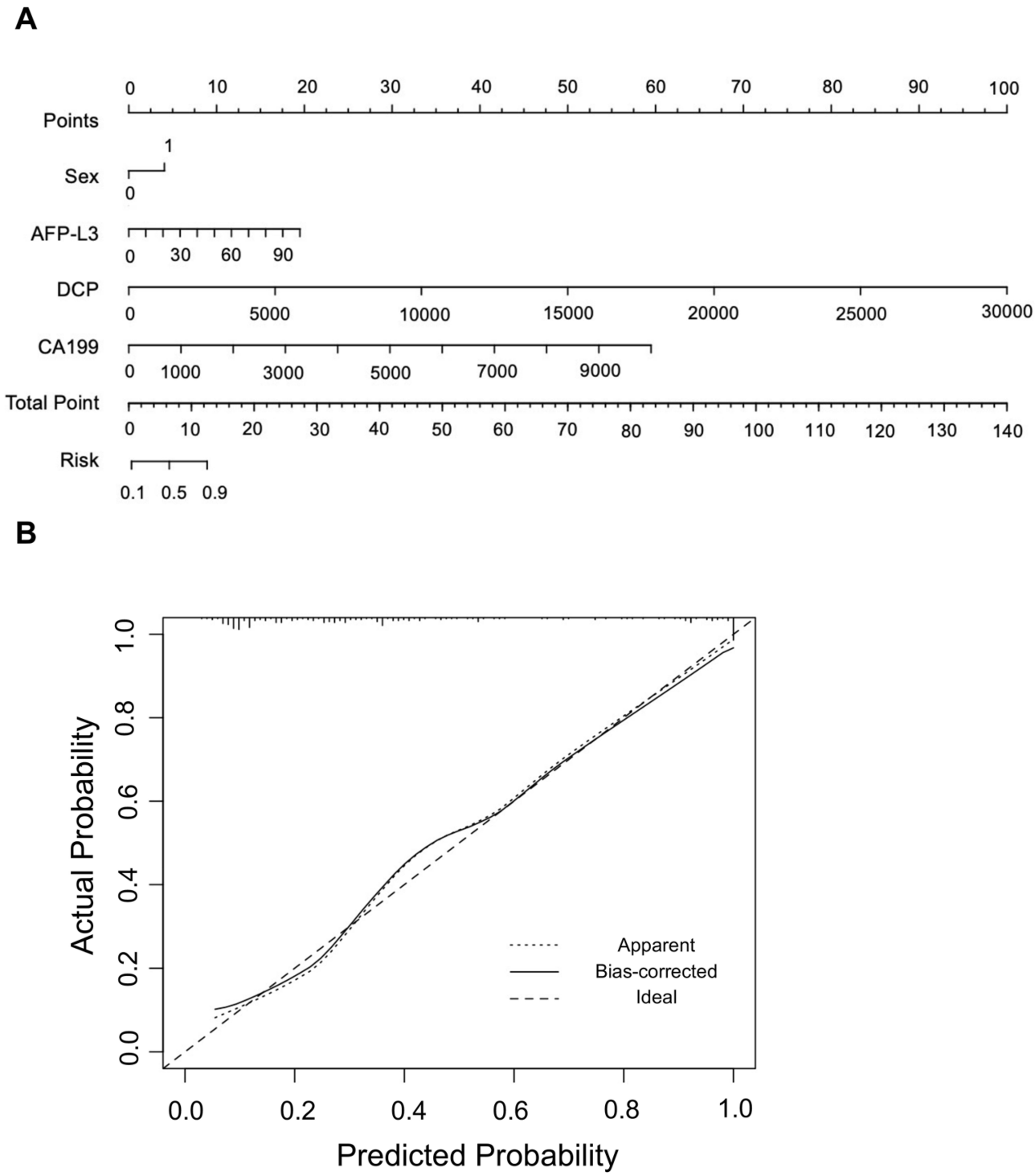

Figure 3 Selected candidate indicators and developed a visualized diagnosis model of PLC. (**A**) The diagnostic nomogram for differentiating PLC from BLD patients. In the sex line, 0 represents female, and 1 represents male. (**B**) The calibration curves for the nomogram. The x-axis represents the nomogram-predicted probability and y-axis represents the actual probability of primary liver cancer.

Despite its strengths, our study has some limitations. It was conducted at a single center with a relatively small sample size, necessitating further validation in external and prospective studies. Additionally, long-term follow-up is required to confirm the diagnostic accuracy of these biomarkers across diverse populations. Future research should also evaluate the cost-effectiveness of combining AFP, AFP-L3, DCP, and CA199 compared to current standards, such as AFP and US.

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Conclusion

In conclusion, our study demonstrates that the combination of AFP, AFP-L3, DCP, and CA199 provides superior diagnostic accuracy compared to the GALAD and ASAP scores for distinguishing PLC patients. These findings suggest that this biomarker panel represents a promising, non-invasive tool for early PLC detection.

Abbreviations

AFP, alpha-fetoprotein; AFP-L3, lens culinaris agglutinin-reactive AFP; ALT, alanine aminotransferase; ALB, albumin; ALP, alkaline phosphatase; AST, aspartate transaminase; ASAP, age, sex, AFP, DCP; CA199, carbohydrate antigen 199; CA242, carbohydrate antigen 242; CA50, carbohydrate antigen 50; CEA, carcinoembryonic antigen; CHC, hepatocellular-cholangiocarcinoma; DB, direct bilirubin; DCP, des-gamma-carboxy prothrombin; GALAD, gender, age, AFP, AFP-L3, and DCP; GGT, gamma-glutamyl transferase; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; MVI, microvascular invasion; NPV, negative predictive value; PLC, primary liver cancer; PLT, platelet; PT, prothrombin time; PPV, positive predictive value; TB, total bilirubin; US, ultrasonography.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Li X, Ramadori P, Pfister D, et al. The immunological and metabolic landscape in primary and metastatic liver cancer. *Nat Rev Cancer*. 2021;21(9):541–557. doi:10.1038/s41568-021-00383-9
2. Sia D, Villanueva A, Friedman SL, et al. Liver cancer cell of origin, molecular class, and effects on patient prognosis. *Gastroenterology*. 2017;152(4):745–761. doi:10.1053/j.gastro.2016.11.048
3. Yang Z, Shi G. Survival outcomes of combined hepatocellular-cholangiocarcinoma compared with intrahepatic cholangiocarcinoma: a SEER population-based cohort study. *Cancer Med*. 2022;11(3):692–704. doi:10.1002/cam4.4474
4. Villanueva A. Hepatocellular Carcinoma. *N Engl J Med*. 2019;380(15):1450–1462. doi:10.1056/NEJMra1713263
5. Piratvisuth T, Tanwandee T, Thongsawat S, et al. Multimarker panels for detection of early stage hepatocellular carcinoma: a prospective, multicenter, case-control study. *Hepatol Commun*. 2022;6(4):679–691. doi:10.1002/hep4.1847
6. McGlynn KA, Petrick JL, El-Serag HB. Epidemiology of hepatocellular carcinoma. *Hepatology*. 2021;73 Suppl 1(Suppl 1):4–13. doi:10.1002/hep.31288
7. Huang C, Xiao X, Zhou L, et al. Chinese expert consensus statement on the clinical application of AFP/AFP-L3%/DCP using GALAD and GALAD-like algorithm in HCC. *J Clin Lab Anal*. 2023;37(23–24):e24990. doi:10.1002/jcla.24990
8. Tzartzeva K, Obi J, Rich NE, et al. Surveillance imaging and alpha fetoprotein for early detection of hepatocellular carcinoma in patients with cirrhosis: a meta-analysis. *Gastroenterology*. 2018;154(6):1706–1718.e1. doi:10.1053/j.gastro.2018.01.064
9. Kim DY, Toan BN, Tan CK, et al. Utility of combining PIVKA-II and AFP in the surveillance and monitoring of hepatocellular carcinoma in the Asia-Pacific region. *Clin Mol Hepatol*. 2023;29(2):277–292. doi:10.3350/cmh.2022.0212
10. Wang H, Lu Z, Zhao X. Tumorigenesis, diagnosis, and therapeutic potential of exosomes in liver cancer. *J Hematol Oncol*. 2019;12(1):133. doi:10.1186/s13045-019-0806-6
11. Qi F, Zhou A, Yan L, et al. The diagnostic value of PIVKA-II, AFP, AFP-L3, CEA, and their combinations in primary and metastatic hepatocellular carcinoma. *J Clin Lab Anal*. 2020;34(5):e23158. doi:10.1002/jcla.23158
12. Feng H, Li B, Li Z, et al. PIVKA-II serves as a potential biomarker that complements AFP for the diagnosis of hepatocellular carcinoma. *BMC Cancer*. 2021;21(1):401. doi:10.1186/s12885-021-08138-3
13. An S, Zhan X, Liu M, et al. Diagnostic and prognostic nomograms for hepatocellular carcinoma based on PIVKA-II and serum biomarkers. *Diagnostics*. 2023;13(8):1442. doi:10.3390/diagnostics13081442
14. Wang MD, Sun LY, Qian GJ, et al. Prothrombin induced by vitamin K Absence-II versus alpha-fetoprotein in detection of both resectable hepatocellular carcinoma and early recurrence after curative liver resection: a retrospective cohort study. *Int J Surg*. 2022;105:106843. doi:10.1016/j.ijsu.2022.106843
15. Yan Q, Sun YS, An R, et al. Application and progress of the detection technologies in hepatocellular carcinoma. *Genes Dis*. 2023;10(5):1857–1869. doi:10.1016/j.gendis.2022.04.003

16. Liu L, Wang Q, Zhao X, et al. Establishment and validation of nomogram model for the diagnosis of AFP-negative hepatocellular carcinoma. *Front Oncol*. 2023;13:1131892. doi:10.3389/fonc.2023.1131892
17. Park SJ, Jang JY, Jeong SW, et al. Usefulness of AFP, AFP-L3, and PIVKA-II, and their combinations in diagnosing hepatocellular carcinoma. *Medicine*. 2017;96(11):e5811. doi:10.1097/MD.0000000000005811
18. Kudo M. Urgent global need for PIVKA-II and AFP-L3 measurements for surveillance and management of hepatocellular carcinoma. *Liver Cancer*. 2024;13(2):113–118. doi:10.1159/000537897
19. Lim TS, Kim DY, Han KH, et al. Combined use of AFP, PIVKA-II, and AFP-L3 as tumor markers enhances diagnostic accuracy for hepatocellular carcinoma in cirrhotic patients. *Scand J Gastroenterol*. 2016;51(3):344–353. doi:10.3109/00365521.2015.1082190
20. Zhang CH, Ni XC, Chen BY, et al. Combined preoperative albumin-bilirubin (ALBI) and serum γ-glutamyl transpeptidase (GGT) predicts the outcome of hepatocellular carcinoma patients following hepatic resection. *J Cancer*. 2019;10(20):4836–4845. doi:10.7150/jca.33877
21. Yang JG, He XF, Huang B, et al. Rule of changes in serum GGT levels and GGT/ALT and AST/ALT ratios in primary hepatic carcinoma patients with different AFP levels. *Cancer Biomark*. 2018;21(4):743–746. doi:10.3233/CBM-170088
22. Best J, Bechmann LP, Sowa JP, et al. GALAD score detects early hepatocellular carcinoma in an international cohort of patients with nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol*. 2020;18(3):728–735.e4. doi:10.1016/j.cgh.2019.11.012
23. Singal AG, Tayob N, Mehta A, et al. GALAD demonstrates high sensitivity for HCC surveillance in a cohort of patients with cirrhosis. *Hepatology*. 2022;75(3):541–549. doi:10.1002/hep.32185
24. Ren T, Hou X, Zhang X, et al. Validation of combined AFP, AFP-L3, and PIVKA II for diagnosis and monitoring of hepatocellular carcinoma in Chinese patients. *Heliyon*. 2023;9(11):e21906. doi:10.1016/j.heliyon.2023.e21906
25. Tayob N, Kanwal F, Alsarraj A, et al. The performance of AFP, AFP-3, DCP as biomarkers for detection of hepatocellular carcinoma (HCC): a Phase 3 biomarker study in the United States. *Clin Gastroenterol Hepatol*. 2023;21(2):415–423.e4. doi:10.1016/j.cgh.2022.01.047
26. Yang T, Xing H, Wang G, et al. A novel online calculator based on serum biomarkers to detect hepatocellular carcinoma among patients with hepatitis B. *Clin Chem*. 2019;65(12):1543–1553. doi:10.1373/clinchem.2019.308965
27. Liu SY, Li C, Sun LY, et al. ASAP score versus GALAD Score for detection of hepatitis C-related hepatocellular carcinoma: a multicenter case-control analysis. *Front Oncol*. 2022;12:1018396. doi:10.3389/fonc.2022.1018396
28. Wakizaka K, Yokoo H, Kamiyama T, et al. Clinical and pathological features of combined hepatocellular-cholangiocarcinoma compared with other liver cancers. *J Gastroenterol Hepatol*. 2019;34(6):1074–1080. doi:10.1111/jgh.14547
29. Kelley RK, Bridgewater J, Gores GJ, et al. Systemic therapies for intrahepatic cholangiocarcinoma. *J Hepatol*. 2020;72(2):353–363. doi:10.1016/j.jhep.2019.10.009
30. Luang S, Teeravirote K, Saentaweesuk W, et al. Carbohydrate antigen 50: values for diagnosis and prognostic prediction of intrahepatic cholangiocarcinoma. *Medicina*. 2020;56(11):616. doi:10.3390/medicina56110616
31. Zhao Y, Zhang C, Zhang Y, et al. Effects of image-guided adaptive radiotherapy combined with hepatic artery chemoembolization in primary liver cancer patients. *Clin Res Hepatol Gastroenterol*. 2022;46(5):101889. doi:10.1016/j.clinre.2022.101889
32. Izquierdo-Sanchez L, Lamarca A, La Casta A, et al. Cholangiocarcinoma landscape in Europe: diagnostic, prognostic and therapeutic insights from the ENSCCA registry. *J Hepatol*. 2022;76(5):1109–1121. doi:10.1016/j.jhep.2021.12.010
33. Moris D, Palta M, Kim C, Allen PJ, Morse MA, Lidsky ME. Advances in the treatment of intrahepatic cholangiocarcinoma: an overview of the current and future therapeutic landscape for clinicians. *CA Cancer J Clin*. 2023;73(2):198–222. doi:10.3322/caac.21759
34. Ma D, Wei P, Liu H, et al. Multi-omics-driven discovery of invasive patterns and treatment strategies in CA19-9 positive intrahepatic cholangiocarcinoma. *J Transl Med*. 2024;22(1):1031. doi:10.1186/s12967-024-05854-9
35. Zeng P, Li H, Chen Y, et al. Serum CA199 levels are significantly increased in patients suffering from liver, lung, and other diseases. *Prog mol Biol Transl Sci*. 2019;162:253–264. doi:10.1016/bs.pmbts.2018.12.010
36. Brown ZJ, Tsilimigras DI, Ruff SM, et al. Management of hepatocellular carcinoma: a review. *JAMA Surg*. 2023;158(4):410–420. doi:10.1001/jamasurg.2022.7989
37. Marrero JA, Kulik LM, Sirlin CB, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. *Hepatology*. 2018;68(2):723–750. doi:10.1002/hep.29913
38. Anwanwan D, Singh SK, Singh S, et al. Challenges in liver cancer and possible treatment approaches. *Biochim Biophys Acta Rev Cancer*. 2020;1873(1):188314. doi:10.1016/j.bbcan.2019.188314
39. Xing X, Cai L, Ouyang J, et al. Proteomics-driven noninvasive screening of circulating serum protein panels for the early diagnosis of hepatocellular carcinoma. *Nat Commun*. 2023;14(1):8392. doi:10.1038/s41467-023-44255-2
40. Norman JS, Li PJ, Kotwani P, et al. AFP-L3 and DCP strongly predict early hepatocellular carcinoma recurrence after liver transplantation. *J Hepatol*. 2023;79(6):1469–1477. doi:10.1016/j.jhep.2023.08.020
41. Hahn M, Herber A, Berg T, et al. Enhancing HCC recurrence prediction after liver transplantation: from DCP plus AFP-L3 model to GALAD score. *J Hepatol*. 2024;80(6):e271–e272. doi:10.1016/j.jhep.2023.12.003
42. Qiu ZC, Wu YW, Qi WL, et al. PIVKA-II combined with tumor burden score to predict long-term outcomes of AFP-negative hepatocellular carcinoma patients after liver resection. *Cancer Med*. 2023;13(1):e6835. doi:10.1002/cam4.6835
43. Liu S, Sun L, Yao L, et al. Diagnostic performance of AFP, AFP-L3, or PIVKA-II for hepatitis C virus-associated hepatocellular carcinoma: a multicenter analysis. *J Clin Med*. 2022;11(17):5075. doi:10.3390/jcm11175075
44. Kong Y, Jing Y, Sun H, et al. The diagnostic value of contrast-enhanced ultrasound and enhanced CT combined with tumor markers AFP and CA199 in liver cancer. *J Healthc Eng*. 2022;2022:5074571. doi:10.1155/2022/5074571
45. Johnson PJ, Pirrie SJ, Cox TF, et al. The detection of hepatocellular carcinoma using a prospectively developed and validated model based on serological biomarkers. *Cancer Epidemiol Biomarkers Prev*. 2014;23(1):144–153. doi:10.1158/1055-9965.EPI-13-0870
46. Cagnin S, Donghia R, Martini A, et al. Galad score as a prognostic marker for patients with hepatocellular carcinoma. *Int J mol Sci*. 2023;24(22):16485. doi:10.3390/ijms242216485