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ORIGINAL RESEARCH

Combined Analysis of Protein Induced by Prothrombin Induced by Vitamin K Absence (PIVKA) and Alpha-L-Fucosidase (AFU) with Alpha-Fetoprotein (AFP) May Improve the Diagnostic Efficacy for Liver Cirrhosis and Hepatocellular Carcinoma in Chronic Hepatitis B

Haisong Hou[1], Ruiqiang Weng[2], Liu Liang[3], Wendao Han[1]

[1]Department of Blood Transfusion, Meizhou People's Hospital, Meizhou Academy of Medical Sciences, Meizhou, People's Republic of China; [2]Institute of Basic Medical Sciences, Meizhou People's Hospital, Meizhou Academy of Medical Sciences, Meizhou, People's Republic of China; [3]Department of Laboratory Medicine, Meizhou People's Hospital, Meizhou Academy of Medical Sciences, Meizhou, People's Republic of China

Correspondence: Haisong Hou, Department of Blood Transfusion, Meizhou People's Hospital, Meizhou Academy of Medical Sciences, Meizhou, People's Republic of China, Email houhaisonggd6@163.com

Background: The aim of this study was to explore the value of biomarkers such as prothrombin induced by vitamin K absence (PIVKA), osteopontin (OPN), α-L-fucoidase (AFU), interleukin-6 (IL-6), and Golgi protein 73 (GP73) in the diagnosis of chronic hepatitis B (CHB), hepatitis B liver cirrhosis (HBV-LC), and hepatocellular carcinoma (HCC).

Methods: A total of 264 male patients were included in this study, including CHB (n=88), HBV-LC (n=88), and HCC (n=88). The levels of PIVKA, OPN, AFU, alpha-fetoprotein (AFP), IL6, and GP73 of the subjects were detected respectively, and the differences in the levels of serum PIVKA, OPN, AFU, AFP, IL6 and GP73 among the groups were compared. The correlations among various indicators were analyzed, and the diagnostic value of these indicators for HBV-LC and HCC was evaluated through the receiver operating characteristic (ROC) curve analysis.

Results: The levels of PIVKA, OPN, AFP, AFU, IL6, and GP73 in patients with HBV-LC, HCC were significantly higher than those in patients with CHB, respectively. In patients with HBV-LC and HCC, no significant correlation was shown between AFP and other markers, suggesting that AFP may have an independent mechanism of action different from other markers in HBV-LC and HCC. OPN had the highest diagnostic efficacy, with an area under the ROC curve (AUC) of 0.855 in diagnosis of HBV-LC, followed by GP73 and IL6. AFU had the highest diagnostic efficacy in diagnosis of HCC, followed by AFP and OPN. In terms of combined detection, the diagnostic efficacy of AFP+AFU (AUC=0.785, 95% CI: 0.714–0.855) and AFP+PIVKA (AUC=0.635, 95% CI: 0.553–0.718) were better than AFP in diagnosing HBV-LC; and the diagnostic efficacy of AFP+AFU (AUC=0.878, 95% CI: 0.822–0.934) was better than AFP in diagnosing HCC.

Conclusion: Combined analysis of PIVKA and AFU with AFP may improve the diagnostic efficacy for HBV-LC and HCC in CHB.

Keywords: chronic hepatitis B, liver cirrhosis, hepatocellular carcinoma, prothrombin induced by vitamin K absence, alpha-L-fucosidase

Introduction

Chronic Hepatitis B (CHB) is a chronic inflammatory disease of the liver caused by persistent infection of hepatitis B virus (HBV).[1,2] According to the definition of the World Health Organization (WHO), when a person is infected with HBV and the disease course exceeds six months, or when HBsAg is positive for more than six months and there are

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manifestations of liver inflammation, it can be diagnosed as CHB.[3,4] Chronic HBV infection is one of the major disease burdens worldwide.[5] HBV is mainly transmitted through blood, mother-to-child, and sexual contact.[6]

Patients with CHB are seriously threatened by CHB liver cirrhosis (HBV-LC) and hepatocellular carcinoma (HCC) due to the progression of the disease.[7,8] Persistent HBV infection triggers chronic inflammation of the liver, activates hepatic stellate cells (HSCs), leads to excessive deposition of extracellular matrix, promotes the progression of liver fibrosis, and eventually develops into liver cirrhosis.[9,10] Epidemiological studies have shown that, approximately over 40% of patients with liver cirrhosis have HBV infection.[11] What is more serious is that HBV-LC is the main risk factor for the occurrence of HCC, and approximately 80% of HCC cases are related to HBV infection.[12,13] Mechanisms such as HBV integration into the host genome,[14] oxidative stress,[15] and DNA damage caused by persistent inflammation[16] promote malignant transformation of hepatocytes.

At present, the early diagnosis, condition monitoring, and prognosis evaluation of HBV-LC and HCC mainly rely on imaging examinations and traditional serological indicators.[17–19] However, imaging examinations have problems such as insufficient sensitivity and difficulty in detecting tiny lesions.[20] Although the traditional marker alpha-fetoprotein (AFP) is widely used in the diagnosis of HCC, both its diagnostic sensitivity and specificity have limitations.[21] Approximately 30% to 40% of patients with early-stage HCC have serum AFP levels that remain within the normal range, which is referred to as AFP-negative HCC.[22] Relying solely on AFP screening will result in the missed diagnosis of approximately one-third of early-stage HCC cases. AFP is not a specific marker for HCC, and it can increase in various benign liver diseases. Clinical data showed that, the false positive rate in benign liver diseases reach 25–30% when the diagnostic threshold is set at AFP >20 ng/mL, indicating its relatively low diagnostic accuracy.[23] Therefore, seeking more sensitive and specific blood-based biomarkers has become the key to improving the diagnosis and treatment level of HBV-LC and HCC.[24]

In recent years, biomarkers such as prothrombin induced by vitamin K absence (PIVKA), osteopontin (OPN), α -L-fucoidase (AFU), interleukin-6 (IL-6), and Golgi protein 73 (GP73) have gradually attracted attention in the research of HBV-LC and HCC. PIVKA is a vitamin K-dependent coagulation factor precursor protein synthesized by the liver. When the liver is damaged, the liver cell's synthesis function declines, and the utilization of vitamin K is impaired, resulting in the accumulation of uncarboxylated PIVKA in the blood. It is an indicator reflecting the liver's synthesis function.[25] OPN is a glycoprotein mainly secreted by liver cells, immune cells and tumor cells, and it has the functions of regulating cell adhesion, migration and inflammatory responses. OPN is involved in the invasion and metastasis of tumor cells and is closely related to the malignancy degree of HCC.[26] AFU is a lysosomal acid hydrolase synthesized and secreted by liver parenchymal cells, and it is involved in the metabolism of glycoproteins and glycolipids. The activity of AFU is significantly increased in HCC.[27] IL-6 is a pleiotropic inflammatory cytokine mainly secreted by mononuclear macrophages, T cells and activated liver cells, it plays a significant role in the occurrence and development of hepatitis B-related liver diseases.[28] GP73 is a transmembrane glycoprotein located in the Golgi apparatus of liver cells. When liver cells are damaged, the expression of GP73 is significantly induced and it is secreted into the blood through the Golgi apparatus, it is upregulated during the progression of liver diseases and has potential value for the early diagnosis of cirrhosis and HCC.[29,30] It is of great significance to deeply explore the clinical application value of these markers in the evaluation of HBV-LC and HCC. Especially in terms of studying the changing patterns (continuously increasing or decreasing) of these indicators during the progression of CHB to liver cirrhosis and HCC, as well as whether the combined detection of AFP and these indicators is superior to the single detection of AFP in the diagnosis of HBV-LC and HCC.

Materials and Methods

Subjects

The subjects of this study were 264 patients who underwent hematological tests and imaging examinations at Meizhou People's Hospital from December 2021 to December 2022. Only male patients were included to avoid sex related biomarker variability. This study included 88 male patients diagnosed with CHB, 88 male patients with HBV-LC, and 88 male patients with HCC. Each participant was informed of the process and purpose of the study. All patients voluntarily

participated in this study and signed the informed consent form. This study complies with the ethical standards stipulated in the Declaration of Helsinki and has been reviewed and approved by the Ethics Committee of Meizhou People's Hospital (Ethics Approval Number: 2021-C-77). The flowchart of this study is shown in Figure 1.

Inclusion criteria: All patients selected in this study were required to meet the diagnostic criteria of CHB,[31,32] specifically:

(1) Patients need to have continuous or regular elevated levels of serum Alanine Aminotransferase (ALT), and the ALT level should reach or exceed more than twice the upper limit of the normal value.
(2) Hepatitis B Surface Antigen (HBsAg) must be detected in the patient's serum, and the presence time of Hepatitis B Virus DNA (HBV DNA) should exceed six months.

Diagnostic Criteria for Diseases

(1) CHB:[31,32]

① Patients need to have continuous or regular elevated levels of serum alanine aminotransferase (ALT), and the ALT level should reach or exceed more than twice the upper limit of the normal value.
② Hepatitis B surface antigen (HBsAg) must be detected in the patient's serum, and the presence time of hepatitis B Virus DNA (HBV DNA) should exceed six months.

(2) HBV-LC:

The diagnostic criteria for hepatitis B cirrhosis: meeting the following A and B (pathological diagnosis), or A and C (clinical diagnosis):

A: the HBsAg positive, or HBsAg negative, HBcAb positive and have clear history of chronic HBV infection (always HBsAg positive > 6 months), and except for other causes.

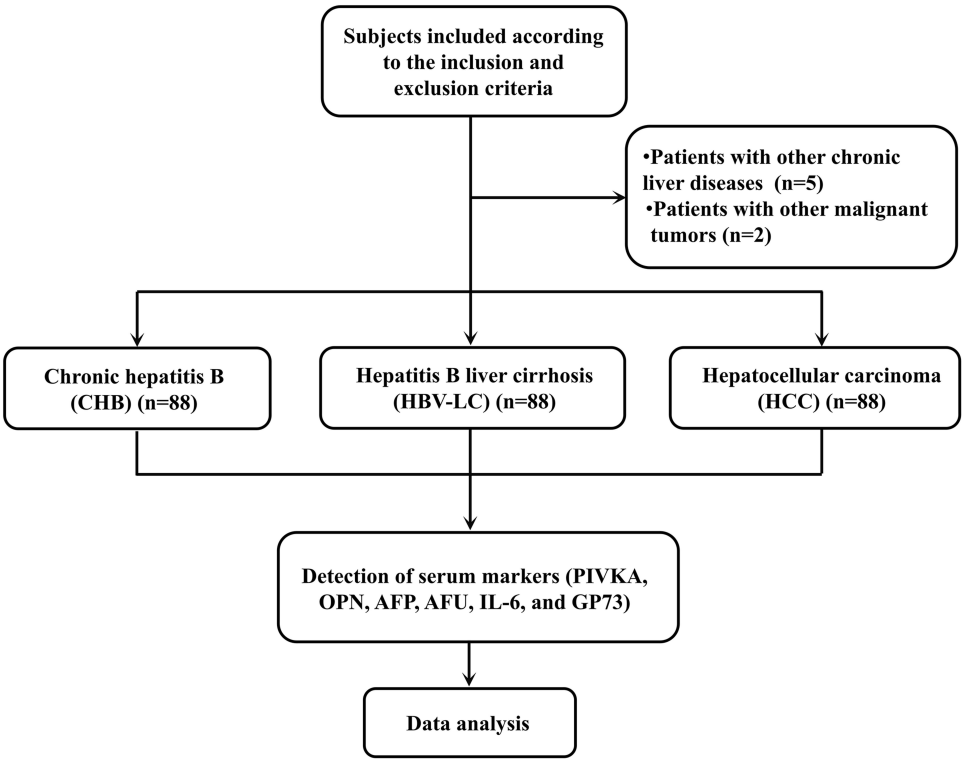

Figure 1 The flow chart of the present study.

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B: the pathology of liver biopsy results accord with cirrhosis performers.

C: conforms to two or more of the following five, and except non liver cirrhosis portal hypertension: ① imaging examination showed signs of liver cirrhosis and portal hypertension (or); ② endoscopy tips for esophageal gastric varices; ③ liver hardness value determination results accord with cirrhosis of the liver; ④ blood biochemical examination results showed that albumin levels (< 35 g/L) and (or) prothrombin time (PT) extension (extended value than control > 3 s); ⑤ the blood routine examination results show that the platelet count < 100×10^9/L.

(3) HCC:

The diagnosis of HCC was based on pathological diagnosis, which served as the gold standard. The pathological diagnosis of HCC is determined through liver biopsy and liver histopathology.[33] When pathological specimens cannot be obtained, clinical diagnosis is made based on the definition of high-risk populations (such as relevant history of liver diseases) and the "rapid enhancement in the arterial phase and rapid attenuation in the portal phase" characteristics of imaging examinations (such as CT/MRI).[34]

Exclusion Criteria

(1) combined with other chronic liver diseases, such as autoimmune liver disease, toxic hepatitis, and so on.
(2) co-infection with other hepatotropic viruses, Human Immunodeficiency Virus (HIV), mental disorders, hematological diseases, and immune system diseases;
(3) pregnant or lactating women;
(4) there is obvious drug-induced liver injury, immune liver disease or other non-viral diseases that may cause liver injury;
(5) HBV-LC or CHB combination with liver space-occupying lesions, or other malignant tumors;
(6) the HCC patients was accompanied by other malignant tumor diseases, there is a previous history of liver cancer, or other malignant tumors, or metastatic liver cancer.

Collection of Serum Samples From Patients and the Detection of Markers

Before the patient received treatment, for each research subject, 5 mL of peripheral blood was collected and placed in a vacuum blood collection tube containing EDTA anticoagulant. The blood was then centrifuged at 3000 rpm for 10 minutes to separate the serum. The serum was immediately stored in a refrigerator at −20°C. The levels of PIVKA, OPN, AFP, AFU, IL-6, and GP73 in the serum of patients were detected by one-step sandwich enzyme-linked immunosorbent assay (ELISA) with double antibodies. All 264 samples were successfully assayed without missing data.

Statistical Analysis

The data were analyzed using the SPSS 26.0 and GraphPad Prism softwares. The measurement data were expressed as medians (25th and 75th percentiles) and compared using Mann–Whitney *U*-test. The correlation among the indicators of PIVKA, OPN, AFP, AFU, IL6, and GP73 were analyzed, and the correlation coefficients (r values) and *p* values were calculated to evaluate the linear relationships among the markers. The specificity and sensitivity of PIVKA, OPN, AFP, AFU, IL6, and GP73 were described using the receiver operating characteristic (ROC) curve analysis. The accuracy of PIVKA, OPN, AFP, AFU, IL6, and GP73 in differentiating HCC and HBV-LC in CHB was evaluated by calculating the area under the ROC curve (AUC), and the optimal cut-off values of PIVKA, OPN, AFP, AFU, IL6, and GP73 were determined using the Youden index. $p<0.05$.

Results

Concentration Differences of Serum Markers Among Different Patients

The concentrations of PIVKA, OPN, AFP, AFU, IL6, and GP73 varied among the three groups of patients. The levels of PIVKA (627.42 (495.31, 721.61) vs 583.62 (376.00, 642.13), $p=0.002$), OPN (1229.78 (1047.69, 1329.92) vs 938.30 (703.75, 1001.20), $p<0.001$), AFP (206.82 (134.29, 226.31) vs 190.11 (157.34, 206.99), $p=0.045$), AFU (363.11 (303.80,

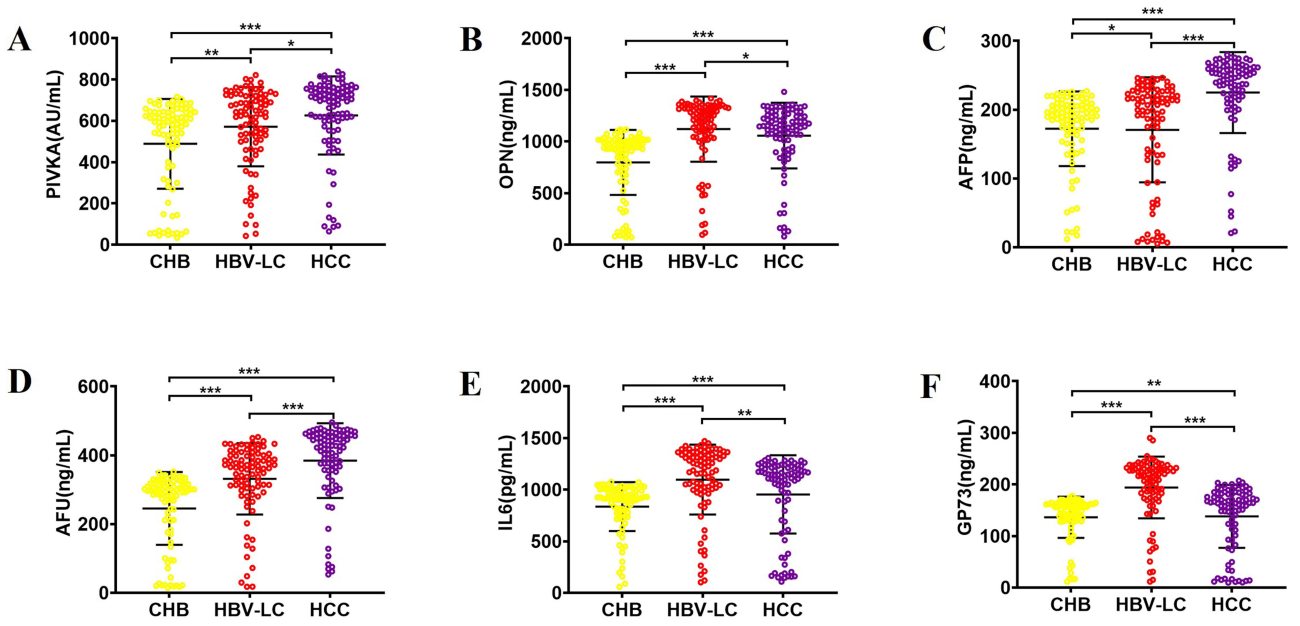

Figure 2 Comparison of levels of PIVKA (**A**), OPN (**B**), AFP (**C**), AFU (**D**), IL-6 (**E**), and GP73 (**F**) in CHB, HBV-LC, and HCC. *p<0.05; **p<0.01; ***p<0.001.
Abbreviations: PIVKA, prothrombin induced by vitamin K absence; OPN, osteopontin; AFP, alpha-fetoprotein; AFU, α -L-fucoidase; IL-6, interleukin-6; GP73, Golgi protein 73; CHB, chronic hepatitis B; HBV-LC, liver cirrhosis; HCC, hepatocellular carcinoma.

399.97) vs 295.11 (210.04, 316.44), p<0.001), IL6 (1198.67 (1003.21, 1342.45) vs 915.78 (771.82, 1007.05), p<0.001), and GP73 (215.22 (174.51, 231.32) vs 150.16 (130.47, 161.46), p<0.001) in patients with HBV-LC were significantly higher than those in patients with CHB (Figure 2).

The levels of PIVKA (695.27 (578.93, 751.66) vs 583.62 (376.00, 642.13), p<0.001), OPN (1145.28 (1010.16, 1255.62) vs 938.30 (703.75, 1001.20), p<0.001), AFP (245.84 (215.33, 261.58) vs 190.11 (157.34, 206.99), p<0.001), AFU (423.79 (356.84, 457.47) vs 295.11 (210.04, 316.44), p<0.001), IL6 (1136.06 (817.48, 1215.58) vs 915.78 (771.82, 1007.05), p<0.001), and GP73 (163.40 (112.02, 181.57) vs 150.16 (130.47, 161.46), p=0.006) in patients with HCC were significantly higher than those in patients with CHB (Figure 2).

Correlation Analysis Among These Markers

In the CHB group, a strong positive correlation was shown between PIVKA and OPN (r=0.9706, p<0.001), PIVKA and AFU (r=0.9840, p<0.001), OPN and AFU (r=0.9876, p<0.001), AFP and IL6 (r=0.9644, p<0.001), AFP and GP73 (r=0.9548, p<0.001), and IL6 and GP73 (r=0.8911, p<0.001), respectively. It suggests that these groups of markers may have a synergistic effect in the pathological process of CHB. It may act jointly in the process of liver inflammatory response and liver function impairment (Figure 3).

In the HBV-LC group, a positive correlation was shown between PIVKA and OPN (r=0.9646, p<0.001), PIVKA and AFU (r=0.9568, p<0.001), PIVKA and IL6 (r=0.9662, p<0.001), PIVKA and GP73 (r=0.9413, p<0.001), OPN and AFU (r=0.9754, p<0.001), OPN and IL6 (r=0.9735, p<0.001), OPN and GP73 (r=0.9709, p<0.001), AFU and IL6 (r=0.9520, p<0.001), AFU and GP73 (r=0.9502, p<0.001), and IL6 and GP73 (r=0.9527, p<0.001), respectively. It suggests that these groups of markers may have a synergistic effect in the pathological process of HBV-LC (Figure 4).

In the HCC group, a positive correlation was shown between PIVKA and OPN (r=0.8590, p<0.001), PIVKA and AFU (r=0.9887, p<0.001), OPN and AFU (r=0.8924, p<0.001), and IL6 and GP73 (r=0.9832, p<0.001), respectively. It indicates that these markers may have a synergistic effect in the pathological process of HCC (Figure 5).

No significant correlation was shown among other markers in each group, indicating that these markers may play a relatively independent role in the progression of patients with CHB. In particular, in patients with HBV-LC and HCC, no significant correlation was shown between AFP and other markers, suggesting that AFP may have an independent mechanism of action different from other markers in HBV-LC and HCC.

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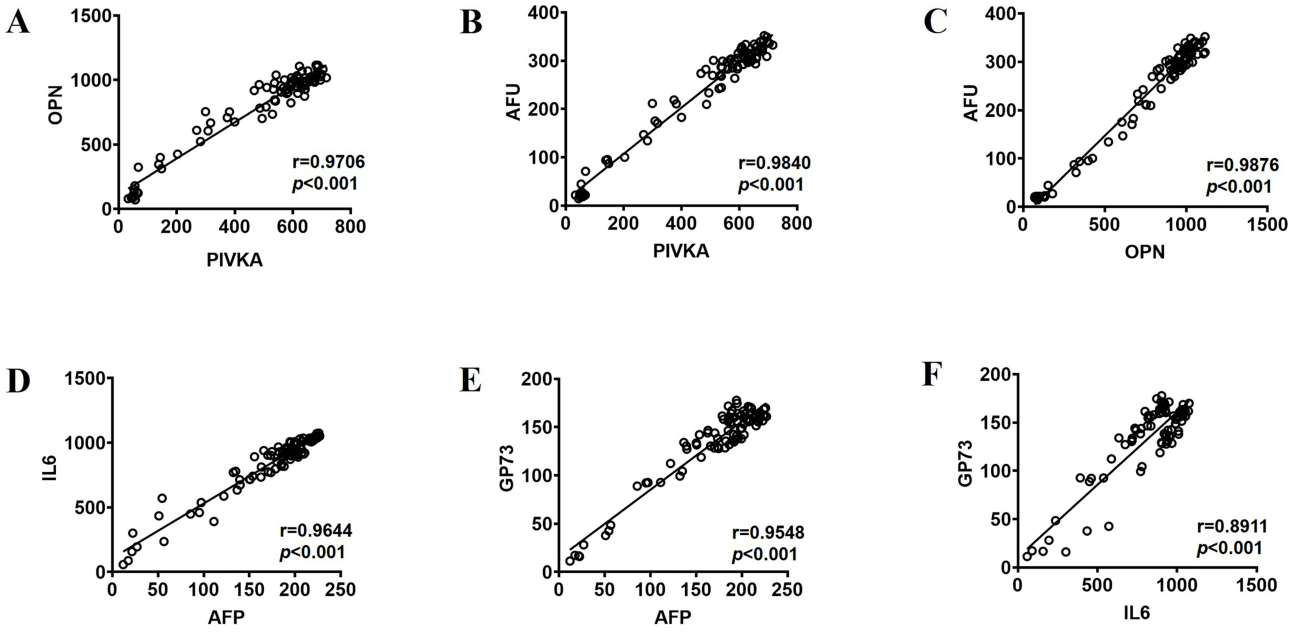

Figure 3 Correlation analysis among PIVKA and OPN (**A**), PIVKA and AFU (**B**), OPN and AFU (**C**), AFP and IL6 (**D**), AFP and GP73 (**E**), and IL6 and GP73 (**F**) in CHB patients.

Abbreviations: PIVKA, prothrombin induced by vitamin K absence; OPN, osteopontin; AFP, alpha-fetoprotein; AFU, α -L-fucoidase; IL-6, interleukin-6; GP73, Golgi protein 73; CHB, chronic hepatitis B.

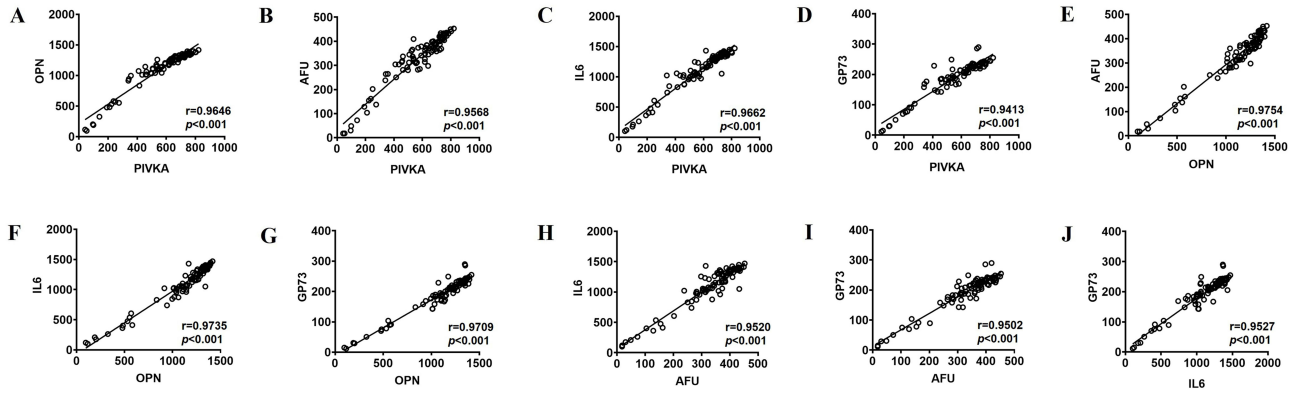

Figure 4 Correlation analysis among PIVKA and OPN (**A**), PIVKA and AFU (**B**), PIVKA and IL6 (**C**), PIVKA and GP73 (**D**), OPN and AFU (**E**), OPN and IL6 (**F**), OPN and GP73 (**G**), AFU and IL6 (**H**), AFU and GP73 (**I**), and IL6 and GP73 (**J**) in HBV-LC patients.

Abbreviations: PIVKA, prothrombin induced by vitamin K absence; OPN, osteopontin; AFU, α -L-fucoidase; IL-6, interleukin-6; GP73, Golgi protein 73; HBV-LC, liver cirrhosis.

The Diagnostic Efficacy of Individual Indicators of These Biomarkers for HBV-LC and HCC Was Evaluated Based on ROC Analysis

In terms of diagnosing HBV-LC, the AUC values of individual markers showed that OPN had the highest diagnostic efficacy, with an area under the ROC curve (AUC) of 0.855 (95% CI: 0.792–0.917)(cut-off value=1039.75, sensitivity=78.4%, specificity=89.8%), followed by GP73 and IL6, with AUC of 0.854 (95% CI: 0.787–0.920)(cut-off value=171.87, sensitivity=78.4%, specificity=97.7%) and 0.812 (95% CI: 0.741–0.883)(cut-off value=1047.97, sensitivity=68.2%, specificity=95.5%). The AUC values of AFU, PIVKA, and AFP were relatively low, which were 0.784 (95% CI: 0.713–0.855)(cut-off value=343.23, sensitivity=59.1%, specificity=97.7%), 0.636 (95% CI: 0.553–0.719)(cut-off value=617.085, sensitivity=55.7%, specificity=65.9%), and 0.587 (95% CI: 0.500–0.675)(cut-off value=206.255, sensitivity=51.1%, specificity=73.9%), respectively (Figure 6A).

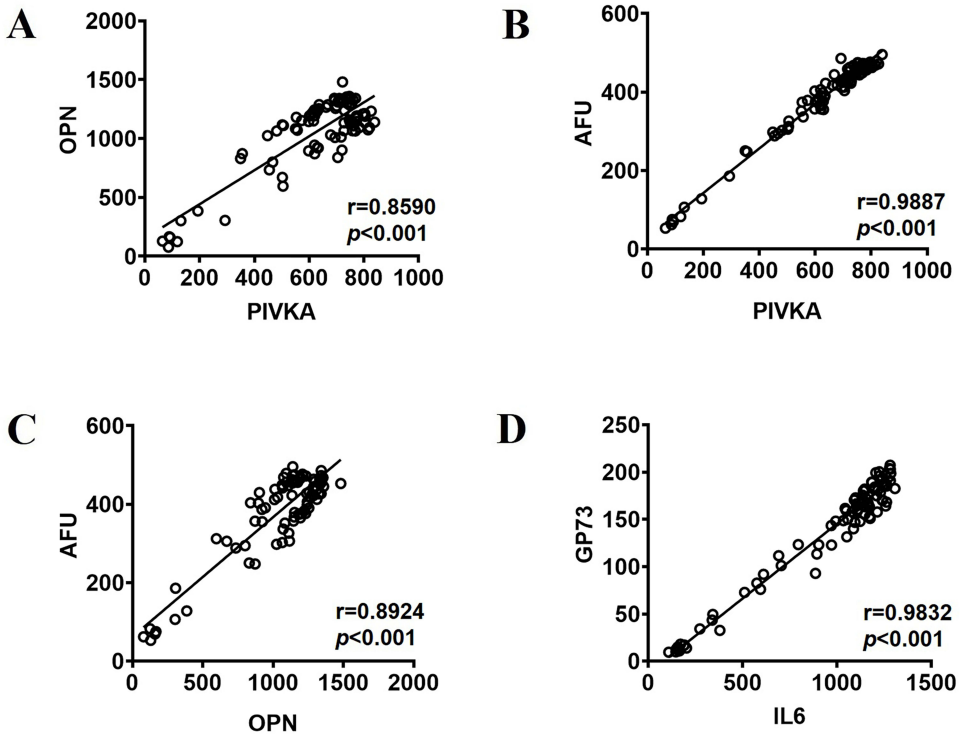

Figure 5 Correlation analysis among PIVKA and OPN (**A**), PIVKA and AFU (**B**), OPN and AFU (**C**), and IL6 and GP73 (**D**) in HCC patients.
Abbreviations: PIVKA, prothrombin induced by vitamin K absence; OPN, osteopontin; AFU, α -L-fucoidase; IL-6, interleukin-6; GP73, Golgi protein 73; HCC, hepatocellular carcinoma.

In terms of diagnosing HCC, the AUC values of individual markers showed that AFU had the highest diagnostic efficacy, with an AUC of 0.873 (95% CI: 0.814–0.931)(cut-off value=334.13, sensitivity=79.5%, specificity=90.9%), followed by AFP and OPN, with AUC of 0.840 (95% CI: 0.775–0.906)(cut-off value=218.18, sensitivity=73.9%, specificity=87.5%) and 0.807 (95% CI: 0.737–0.877)(cut-off value=1059.155, sensitivity=71.6%, specificity=92.0%). The AUC values of PIVKA, IL6, and GP73 were relatively low, which were 0.742 (95% CI: 0.668–0.816)(cut-off value=689.025, sensitivity=53.4%, specificity=94.3%), 0.737 (95% CI: 0.652–0.821)(cut-off value=1040.06, sensitivity=67.0%, specificity=93.2%), and 0.619 (95% CI: 0.531–0.707)(cut-off value=163.31, sensitivity=51.1%, specificity=83.0%), respectively (Figure 6B).

The Diagnostic Efficacy of the Combined Detection of AFP and Other Indicators for HBV-LC and HCC Was Evaluated Based on ROC Analysis

In terms of diagnosing HBV-LC, AFP + OPN had the highest diagnostic efficacy (AUC=0.856, 95% CI: 0.793–0.918)(sensitivity=78.4%, specificity=92.0%), followed by AFP + GP73 (AUC=0.849, 95% CI: 0.781–0.917)(sensitivity=78.4%, specificity=95.5%), AFP + IL6 (AUC=0.809, 95% CI: 0.736–0.883)(sensitivity=75.0%, specificity=88.6%), AFP + AFU (AUC=0.785, 95% CI: 0.714–0.855)(sensitivity=61.4%, specificity=94.3%), and AFP + PIVKA (AUC=0.635, 95% CI: 0.553–0.718)(sensitivity=46.6%, specificity=22.7%) (Figure 6C).

In terms of diagnosing HCC, AFP + AFU had the highest diagnostic efficacy (AUC=0.878, 95% CI: 0.822–0.934)(sensitivity=77.3%, specificity=97.7%), followed by AFP + IL6 (AUC=0.833, 95% CI: 0.768–0.897)(sensitivity=69.3%, specificity=93.2%), AFP + GP73 (AUC=0.833, 95% CI: 0.765–0.901)(sensitivity=73.9%, specificity=93.2%), AFP + OPN (AUC=0.829, 95% CI: 0.765–0.894)(sensitivity=73.9%, specificity=88.6%), and AFP + PIVKA (AUC=0.825, 95% CI: 0.760–0.889)(sensitivity=71.6%, specificity=89.8%) (Figure 6D).

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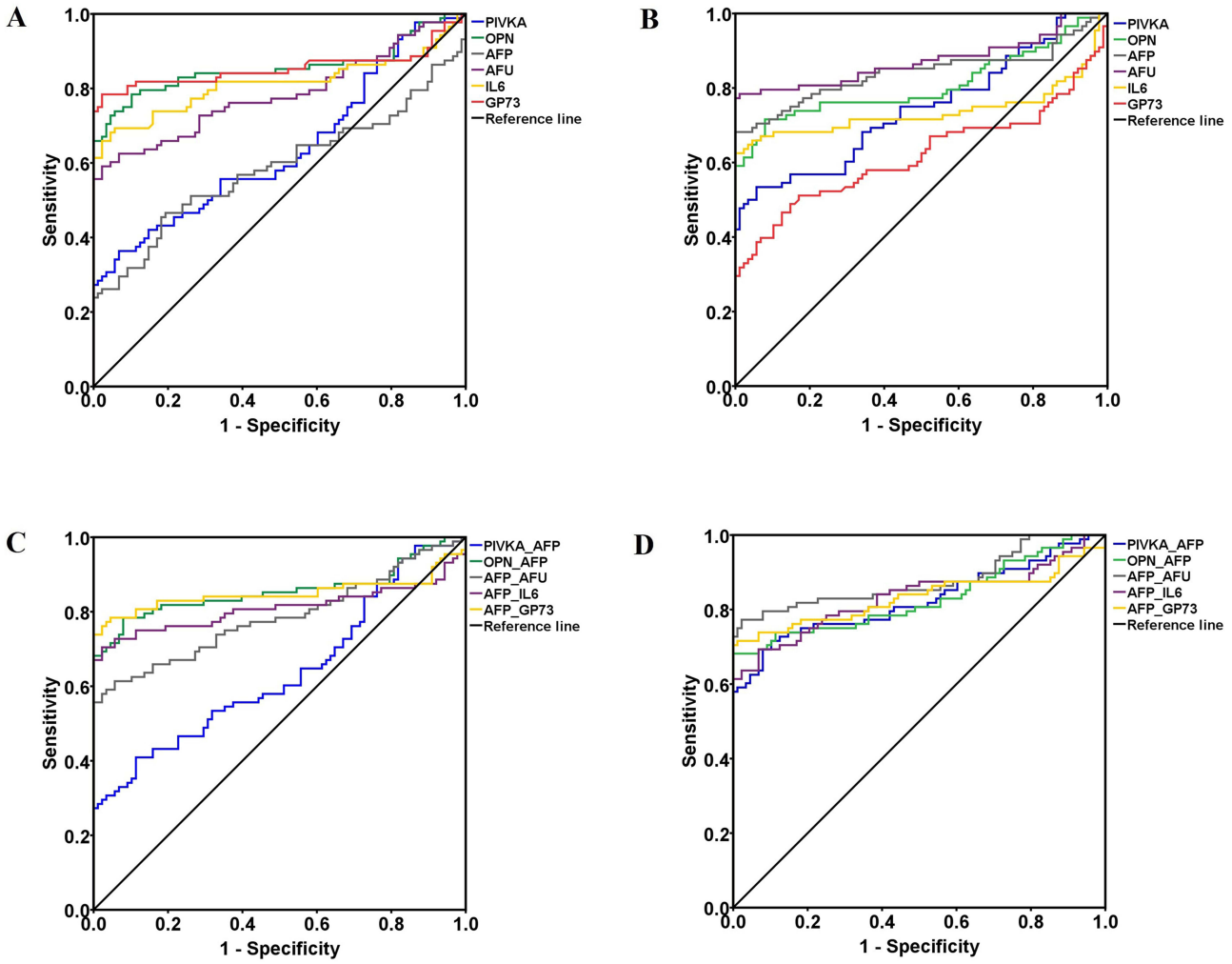

Figure 6 Diagnostic efficacy of PIVKA, OPN, AFP, AFU, IL-6, and GP73 alone for HBV-LC (**A**) and HCC (**B**) respectively, and the combined detection of AFP and other indicators for HBV-LC (**C**) and HCC (**D**) respectively, based on ROC analysis.

Abbreviations: PIVKA, prothrombin induced by vitamin K absence; OPN, osteopontin; AFP, alpha-fetoprotein; AFU, α -L-fucoidase; IL-6, interleukin-6; GP73, Golgi protein 73; HBV-LC, liver cirrhosis; HCC, hepatocellular carcinoma.

Discussion

At present, more and more studies are focusing on the detection of serum markers of HCC. AFP, as a classic tumor marker for liver cancer, although its sensitivity and specificity are limited in patients with early HCC, the combination of detection by other indicators may significantly improve the accuracy of diagnosis.[35,36] This study evaluated the levels of PIVKA, OPN, AFP, AFU, IL-6, and GP73 in patients with CHB, HBV-LC, and HCC, aiming to explore their roles in the diagnosis of HBV-LC and HCC in CHB. The results showed that combined analysis of PIVKA and AFU with AFP may improve the diagnostic efficacy for HBV-LC and HCC in CHB.

Liver is the main site for the synthesis of vitamin K-dependent proteins.[37] Persistent chronic hepatitis B virus infection can trigger an inflammatory response in the liver, causing damage to liver cells and hindering the processes of vitamin K absorption, transport and utilization.[38] In this case, the liver is unable to carboxylate vitamin K-dependent proteins normally, thereby generating a large amount of PIVKA.[39] For patients with liver cirrhosis, liver tissue fibrosis and pseudolobule formation further disrupt the normal structure and function of the liver, aggravate the disorder of vitamin K metabolism, and lead to an increase in PIVKA levels.[25] During the occurrence and development of HCC, tumor cells themselves can abnormally express γ-glutamyl carboxylase, promoting an increase in the production of uncarboxylated proteins.[40] At the same time, tumor growth compresses the intrahepatic blood vessels, affecting the

supply and metabolism of vitamin K, may lead to a significant increase in PIVKA level.[41] In this study, compared with the traditional diagnostic indicator AFP, PIVKA showed good diagnostic efficacy for HBV-LC. The combination of AFP and PIVKA detection can effectively improve the diagnostic rate. The combined detection of PIVKA and AFP can achieve complementary advantages and significantly improve the accuracy and reliability of HBV-LC diagnosis. It is consistent with the results of previous studies.[25,42–44]

In this study, AFU showed a significant increase in the serum of patients with HBV-LC and HCC, which was consistent with some previous research results.[27,43,45] AFU, as a lysosomal acid hydrolase, is involved in the metabolic processes of glycoproteins and glycolipid.[46] During the progression of CHB to HBV-LC, liver cells are continuously damaged and repaired, liver tissue becomes fibrotic, and cell metabolism is disordered, resulting in an increase in the synthesis and release of AFU.[47] The data of this study showed that the serum AFU level of patients in the liver cirrhosis group was significantly higher than that in the chronic hepatitis B group, indicating that AFU can serve as a potential biomarker reflecting the progression of CHB to HBV-LC stage. For HCC, the diagnostic value of AFU is more prominent. Studies have found that the serum AFU level of patients with HCC is not only significantly higher than that of the CHB and liver cirrhosis groups, but also has a higher diagnostic efficacy compared with AFP.[27,45] The combined detection of AFU and AFP can significantly improve the diagnostic efficacy for HCC.

The results of this study show that AFP, PIVKA, and AFU have a gradually increasing trend in patients with CHB, HBV-LC, and HCC. Meanwhile, OPN, IL-6, and GP73 have the highest levels in the HBV-LC group. This difference may be closely related to the differences in pathological characteristics, biological functions of markers, and the relationship of pathological processes at different disease stages. For AFP, PIVKA, and AFU, the expressions of all three are closely related to the proliferation activity and differentiation status of liver cells. AFP, as a classic embryonic protein, shows a continuous increase in synthesis as the tumor progresses.[48] In HCC cells, metabolic disorders of vitamin K can lead to a significant accumulation of PIVKA, the more active the tumor cells are, the higher the expression of PIVKA will be.[49–51] The high metabolic activity of HCC cells and abnormal glycosylation modification will stimulate the synthesis and release of AFU, and at the same time, the invasion of tumor tissues into surrounding normal liver cells will also lead to an increase in the release of enzymes into the blood.[27] Therefore, from the mild liver cell damage in CHB, to the compensatory regeneration of liver cells in HBV-LC, and to the malignant proliferation of HCC, the expressions of AFP, PIVKA, and AFU increase successively as the upgrade of liver cell proliferation activity and abnormal differentiation degree, which conforms to the correlation pattern between tumor markers and malignant cell phenotypes.

The levels of OPN, IL-6, and GP73 were the highest in the HBV-LC group. The reason for it might lie in the fact that the biological functions of these three factors are highly consistent with the pathological process of liver fibrosis remodeling and chronic inflammation activation. OPN can regulate extracellular matrix (ECM) remodeling and fibroblast activation.[52] During the HBV-LC stage, the synthesis of OPN increases, but when the disease progresses to HCC, the liver fibrosis process has become relatively stable, so its level is lower than that in the HBV-LC group. In the HBV-LC stage, IL-6 promotes hepatocyte apoptosis and activation of HSCs, exacerbating fibrosis.[53,54] When it progresses to HCC, although tumor cells secrete IL-6 to regulate the immunosuppressive microenvironment, the level of IL-6 is lower than that in the HBV-LC stage. During the HBV-LC stage, the repeated necrosis and regeneration of liver cells lead to dysfunction of the Golgi apparatus, and a large amount of GP73 is released into the blood.[55,56] In the HCC stage, although there is abnormal proliferation of liver cells, the structure and function of the Golgi apparatus of tumor cells are more inclined to support tumor metabolism. At this time, the intensity of the cycle of injury-repair within the liver is weaker than that in the HBV-LC stage, so the level of GP73 reaches the highest in the HBV-LC group.

Although this research yielded some valuable results, it still has certain limitations. First, the sample size included in this study was relatively small, and the diagnostic efficacy analysis was based solely on internal cases from a single center. No multi-center external validation trials were conducted, and the diagnostic stability of these indicators in different clinical scenarios was not confirmed. This limited the generalizability of the study results and the feasibility of their application in clinical practice. Second, this study only explored the value of these indicators in the diagnosis of HBV-LC and HCC, and did not conduct in-depth research on their relationship with the severity of HBV-LC and HCC. Third, due to the small number of cases included in the study, this research was unable to examine the influence of other factors (such as age, gender, comorbidities, and so on) on these indicators, which limits the generalizability of the

research results. Furthermore, this study has not conducted in-depth research on the dynamic changes of these indicators during the disease treatment process and their relationship with the treatment effect. In the future, it is necessary to further clarify their clinical significance in the diagnosis and treatment of HBV-LC and HCC.

It is worth noting that currently, there are relatively few academic studies on the reduction of PIVKA and AFU levels in the diagnosis of HBV-LC and HCC, and the related mechanisms have not been fully clarified. This study only preliminarily reveals the potential significance of the levels of both in the diagnosis of HBV-LC and HCC. Future research can be conducted from several aspects. First, establish a standardized detection and evaluation system that combines PIVKA, AFU, and AFP. Second, conduct prospective cohort verification on the sensitivity of the combined detection model in early HCC cases with AFP <20 ng/mL. Third, combine the detection model with radiomics to enhance the detection ability for sub-centimeter lesions.

Conclusion

In present study, AFP, PIVKA-II, and AFU show a gradually increasing trend in patients with CHB, HBV-LC, and HCC. Combined analysis of PIVKA and AFU with AFP may improve the diagnostic efficacy for HBV-LC and HCC in CHB. That is to say, compared with a single detection index, the comprehensive analysis of PIVKA, AFU and AFP can be regarded as an optimal laboratory testing plan for the diagnosis and screening of advanced-stage CHB in clinical practice. Of course, it requires more subsequent research to confirm.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of Medicine, Meizhou People's Hospital. All participants signed informed consent in accordance with the Declaration of Helsinki.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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