The clinical application of PIVKA-II in hepatocellular carcinoma and chronic liver diseases: A multi-center study in China

Jun Ji1 | Lijuan Liu2 | Feifei Jiang3 | Xue Wen1 | Yu Zhang2 | Shengcong Li2 | Jinli Lou3 | Ying Wang3 | Ning Liu3 | Qiuyan Guo3 | Yongmei Jia3 | Chunfang Gao1

1Department of Laboratory Medicine, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai, China
2Department of Laboratory Medicine, Mengchao hepatobiliary Hospital of Fujian Medical University, Fujian, China
3Center for Clinical Laboratory, Beijing Youan Hospital, Capital Medical University, Beijing, China

Abstract

Background: Due to the absence of specific symptoms and low survival rate, efficient biomarkers for hepatocellular carcinoma (HCC) diagnosis are urgently required. The purpose of this study was to evaluate the diagnostic performance of protein induced by vitamin K absence or antagonist-II (PIVKA-II) and to determine the optimal cutoff values for HBV infection-related HCC.

Methods: We conducted a cross-sectional, multi-center study in China to ascertain the cutoff value for HCC patients in the context of CHB- and HBV-related cirrhosis. The receiver operating characteristic curve (ROC) and the area under the curve (AUC) were used to evaluate the diagnostic performance of PIVKA-II.

Results: This study enrolled 784 subjects and demonstrated that PIVKA-II had a sensitivity of 84.08% and a specificity of 90.43% in diagnosis HCC from chronic liver diseases. PIVKA-II at a cutoff of 37.5 mAU/mL yielded an AUC of 0.9737 (sensitivity 91.78% and specificity 96.30%) in discriminating HCC from chronic hepatitis B (CHB) patients. PIVKA-II at a cutoff of 45 mAU/mL yielded an AUC of 0.9419 (sensitivity 77.46% and specificity 95.12%) in discriminating HCC- from HBV-related cirrhosis patients. Furthermore, using a cutoff value of 40 mAU/mL for PIVKA-II as an HCC marker, only 4.81% (15/312) was positive in chronic hepatitis and 12.80% (37/289) in cirrhosis patients, revealing the satisfactory specificity of PIVKA-II in chronic liver disease of different etiologies.

Conclusion: Our data indicated that PIVKA-II had satisfactory diagnostic efficiencies and could be used as a screening or surveillance biomarker in HCC high-risk population.

KEYWORDS
chronic liver diseases, diagnostic evaluation, hepatocellular carcinoma, multi-center study, Protein induced by vitamin K absence or antagonist-II
1 | INTRODUCTION

In 2018, liver cancer ranked the sixth most common cancer and the fourth leading cause of cancer deaths in the worldwide. Every year, there are about 841,000 new cases and 782,000 deaths globally. Primary liver cancer can be divided into hepatocellular carcinoma (HCC), cholangiocarcinoma (ICC), and mixed type according to the pathological types, while HCC accounts for about 90% of all cases. As a high-risk area of HCC, China contributes to more than half of the new incidence and mortality related to HCC annually, and the morbidity and mortality rates ranked the fourth and third in China, respectively. Due to the absence of specific symptoms and early diagnosis, 70%-80% of patients are often diagnosed at an advanced stage with a 5-year survival rate less than 40% and a recurrence rate exceeding 60%. Thus, effective tools for HCC diagnosis and prognosis prediction are urgently required.

Although there are regional differences, the main risk factors for HCC include virus infection (hepatitis B virus [HBV], hepatitis C virus [HCV]), aflatoxin-contaminated foodstuffs, alcoholism, obesity, and type 2 diabetes. HBV contributes the largest proportion to liver cancer mortality in East Asia, at 41%. The current HBV infection rate in China is 7.8%, exceeding 60%. Thus, effective tools for HCC diagnosis and prog -

2 | MATERIALS AND METHODS

2.1 | Study design and participants

This was a cross-sectional, multi-center study. The study protocol was approved by the institutional review board of Eastern Hepatobiliary Surgery Hospital Affiliated to The Second Military Medical University (Cohort A), Beijing Youan Hospital (Cohort B), and Mengchao Hepatobiliary Hospital of Fujian Medical University (Cohort C). All the samples were leftover samples, and informed consent process was not required for this type of samples according to institutional review board approval. A total of 784 subjects from the above three hospitals were enrolled in this study from October 2017 to September 2018. Thereinto, 183 cases were liver cancer samples, 312 cases were chronic hepatitis, and 289 cases were cirrhosis. All the clinical diagnostic criteria for each disease were followed the Chinese-related guidelines.

All the study subjects met the following criteria: Chinese, age 18 and older, and regardless of gender. Exclusion criteria were as follows: acute hepatitis of any cause; concomitant HIV or other autoimmune diseases; patients received warfarin or vitamin K prior to testing; specimen contaminated or with sediment or flocculation; samples with insufficient volume; contrary to the diagnostic criteria of the Chinese-related guidelines.

2.2 | Specimen collection and testing methods

The serum samples were collected at the time of diagnosis without treatment and stored at -20°C or lower temperature immediately. PIVKA-II concentrations were measured by the chemiluminescence enzyme immunoassay (CLEIA) (Lumipulse G1200, Fujirebio, Tokyo, Japan) and the chemiluminescent microparticle immunoassay (CMA) (Architect i2000, Abbott Diagnosis, Abbott Park, America) following the manufacturer's protocol, respectively.

2.3 | Performance evaluation

2.3.1 | Reproducibility

Four concentration sample pools including low concentration (≤40 mAU/mL), medium concentration (41~200 mAU/mL), high concentration (201~1000 mAU/mL), and super-high concentration (1001~30,000 mAU/mL) were prepared and measured ten times on the first day to calculate their coefficient of variation (CV%) at three research centers respectively. The 4-level samples were measured six times again...
on days 3 and 7 after storage at 4°C; and days 14 and 30 after storage at −20°C to satisfy the different storage needs of clinical samples.

### 2.3.2 Dilution linearity

Fourteen high concentration (20,000–30,000 mAU/mL) sample pools were prepared to evaluate linearity of the assay. Double dilution methods were performed by diluting specimens (with the specimen diluent of Lumipulse or the Calibrator A (0 mAU/mL) of Architect) until 1:32 dilution. Linearity was evaluated as the coefficient of determination ($R^2$) between measured and expected values for the respective samples.

### 2.4 Statistical analysis

All data were analyzed by GraphPad Prism 6 (GraphPad Software, La Jolla, CA) and Medcalc (MedCalc, Ostend, Belgium) software programs. The Mann-Whitney U test was used for the differential analysis of PIVKA-II concentration. Discrete variables were compared by contingency table analysis of the chi-square test or Fisher’s Exact Test. Correlation coefficients were calculated to observe the correlation between two assays. The receiver operating characteristic curve (ROC) and the area under the curve (AUC) were used to evaluate the diagnostic performance of PIVKA-II. The optimal cutoff value was determined by Youden’s index. Two-sided settings were used for statistical analysis and $p \leq 0.05$ was considered as statistically significant.

### 3 RESULTS

#### 3.1 Participates characteristics

As shown in Figure 1, 950 individuals were included in the study, and 166 were excluded due to the incomplete information or other malignancies. Eventually, 784 participates were enrolled in the study. The basic characteristics of every participant were summarized in Table 1. Patients with liver cancer and cirrhosis were predominantly male ($p < 0.0001$), while the gender difference was not significant in patients with chronic hepatitis. Simultaneously, patients with liver cancer and cirrhosis were older than those with chronic hepatitis ($p < 0.0001$). According to the results of previous large-scale studies, the concentrations of PIVKA-II have no relationship with age or gender, and thus, we did not conduct further statistical processing on the samples. In HCC group, 91.7% cases had HBV infection, 3.18% cases had HCV infection, and 5.1% cases had other pathogeneses.

![Figure 1](image-url)  
**Figure 1** Study profile. Cohort A, Eastern Hepatobiliary Surgery Hospital; Cohort B, Beijing Youan Hospital; Cohort C, Mengchao Hepatobiliary Hospital of Fujian Medical University; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; MT, liver metastasis; CH, chronic hepatitis; LC, liver cirrhosis
The concentrations of PIVKA-II were significantly higher in HCC group (median 466 mAU/mL, 95% CI 194–841) than other liver cancers (ICC: median 27 mAU/mL, 95% CI 24–36, p < 0.0001; MT: median 23 mAU/mL, 95% CI 21–26, p < 0.0001) and non-HCC disease control (DC) groups (CHS: median 20 mAU/mL, 95% CI 19–22, p < 0.0001; liver cirrhosis [LC] median 17 mAU/mL, 95% CI 15–19.97, p < 0.0001). Consistent with previous studies, our data revealed that the sensitivity of PIVKA-II in ICC was 21.05%.25

3.2 | Satisfactory diagnostic performance of PIVKA-II

As shown in Figure 2 and Table 2, PIVKA-II had a sensitivity of 84.08% and a specificity of 90.43% when distinguishing HCC from disease controls at the cutoff value of 40 mAU/mL; a sensitivity of 84.08% and a specificity of 94.55% when distinguishing HCC from patients with different etiology of chronic hepatitis; and a sensitivity of 84.08% and a specificity of 86.51% when distinguishing HCC from cirrhosis. As shown in Figure 3, the correlation coefficient of two PIVKA-II assays was 0.9869. For the samples in various concentration ranges, the Lumipulse reagent showed the satisfactory reproducibility, regardless of stored in room temperature (RT) or different storage conditions. The CV values for all the concentrations and the conditions from three research centers were documented in Table 3. Figure 4 and Table 4 evaluated the dilution linearity of PIVKA-II reagents. The coefficient of determination ($R^2$) between the measured values and the expected values of all the samples was 0.9917–0.9999 according to the Lumipulse’s data.

3.3 | The distribution of PIVKA-II among chronic liver diseases

To better explore the specificity of PIVKA-II, we observed the distribution of PIVKA-II among chronic liver diseases with different etiologies in Figure 5. The mean value of PIVKA-II in CH was 25.04 mAU/mL (range 2–297 mAU/mL), and the value of PIVKA-II in chronic hepatitis B (mean 20.83 mAU/mL, range 6–61 mAU/mL) was close to it. Notably, the concentrations of PIVKA-II in CHC were significantly lower (mean 12.83 mAU/mL, range 2–40 mAU/mL). Among CH, ASH had the highest concentration (mean 48.80 mAU/mL, range 12–296 mAU/mL). There is no doubt that the concentrations of PIVKA-II in LC group (mean 56 mAU/mL, range 2–3057 mAU/mL) were comparatively higher than the CH group. AC also revealed the higher concentration (mean 101.7 mAU/mL, range 2–3057 mAU/
mL) among the LC group. Above all, the positive rate of PIVKA-II exceeding 40 mAU/mL cutoff value was 4.81% in 312 CH patients and 12.8% in 289 LC patients, respectively.

### 3.4 The optimal cutoff values of PIVKA-II for diagnosing HCC in patients with CHB or cirrhosis

Since the key determinant of HCC in China is HBV infection, HCC surveillance in patients with these contexts was significantly important. Therefore, we inquired the optimal cutoff values of PIVKA-II in patients with CHB- or HBV-related cirrhosis (Figure 6). PIVKA-II at a cutoff value of 37.5 mAU/mL yielded an AUC of 0.9737, with a sensitivity of 91.78% and a specificity of 96.3%, in discriminating HBV-related HCC patients without cirrhosis from CHB. PIVKA-II at a cutoff value of 45 mAU/mL yielded an AUC area of 0.9419, with a sensitivity of 77.46% and a specificity of 95.12%, in discriminating HBV-related HCC with cirrhosis from HBV-related cirrhosis patients.

### 4 DISCUSSION

As the most widely used HCC marker, AFP always has a false-negative rate from 30% to 40%, and its concentrations may be elevated in several nonspecific conditions other than HCC. After AFP, many studies have revealed that elevated PIVKA-II is associated with tumor size and vascular invasion and has highly sensitivity...
and specificity for the diagnosis and prognosis monitoring of HCC, especially in HBV-related liver disease. In this study, we investigated the diagnostic efficacy of PIVKA-II in patients with different etiologies of chronic hepatitis and cirrhosis. The results showed that PIVKA-II had a sensitivity of 84.08% and a specificity of 94.55% when distinguishing HCC from patients with different etiology of chronic hepatitis; a sensitivity of 84.08% and a specificity of 86.51% when distinguishing HCC from cirrhosis. In accordance with previous reports, these data indicated the excellent diagnostic efficacy of PIVKA-II in liver cancer. Considering that AFP and PIVKA-II are not correlated, the combination of AFP and PIVKA-II measurement will have better diagnostic performances on HCC. Our data also validated that the combination of AFP and PIVKA-II increases the detection rate of HCC.

The recent global burden of primary liver cancer has indicated that HBV was the leading cause of liver cancer occurrence and death, followed by HCV and alcohol. CHB patients without antiviral treatment may progress to severe fibrosis and cirrhosis at a rate of 2%–10% and the incidence of HCC in patients with cirrhosis is 3%–6%. On the other hand, in non-cirrhotic CHB patients, there is still 0.5%–1.0% incidence of HCC. A multi-center investigation conducted by 112 hospitals in China enrolled 18,275 HCC patients without antiviral treatment. Among them, 3% of patients developed HCC within 5 years, and 6% of patients developed HCC within 10 years.

### TABLE 3 Reproducibility data of four concentration samples at different conditions measured by two PIVKA-II assays

| Concentration | Institution | RT | 4°C 3d | 4°C 7d | −20°C 14d | −20°C 30d |
|---------------|-------------|----|--------|--------|-----------|-----------|
| Lumipulse     | Cohort A    | 6.03% | 5.27% | 5.87% | 3.18% | 4.02% |
|               | Cohort B    | 2.65% | 6.56% | 2.99% | 2.50% | 3.16% |
|               | Cohort C    | 3.76% | 3.76% | 6.88% | 5.11% | 2.43% |
|               | Cohort A    | 3.37% | 2.59% | 2.36% | 3.13% | 2.29% |
|               | Cohort B    | 3.57% | 3.86% | 1.42% | 2.76% | 3.03% |
|               | Cohort C    | 2.95% | 2.66% | 1.49% | 2.91% | 2.02% |
|               | Cohort A    | 1.82% | 2.01% | 2.32% | 2.24% | 2.98% |
|               | Cohort B    | 2.44% | 3.30% | 1.23% | 2.48% | 2.25% |
|               | Cohort C    | 1.44% | 1.51% | 1.88% | 2.35% | 3.06% |
|               | Cohort A    | 2.34% | 5.03% | 2.08% | 1.86% | 3.39% |
|               | Cohort B    | 1.13% | 3.95% | 1.17% | 2.64% | 1.34% |
|               | Cohort C    | 2.13% | 1.96% | 1.82% | 2.08% | 1.92% |
| Architect     | Cohort A    | 5.02% | 5.96% | 5.89% | 3.25% | 3.39% |
|               | Cohort B    | 3.42% | 2.68% | 2.47% | 3.82% | 2.71% |
|               | Cohort C    | 5.31% | 9.85% | 9.18% | 5.69% | 10.29% |
|               | Cohort A    | 4.54% | 2.78% | 2.96% | 3.51% | 3.08% |
|               | Cohort B    | 3.44% | 3.49% | 4.56% | 3.89% | 2.66% |
|               | Cohort C    | 6.34% | 4.27% | 5.61% | 11.53% | 4.05% |
|               | Cohort A    | 2.11% | 1.90% | 2.95% | 2.28% | 4.58% |
|               | Cohort B    | 3.16% | 2.65% | 5.05% | 2.68% | 2.50% |
|               | Cohort C    | 4.27% | 5.46% | 4.80% | 7.07% | 1.81% |
|               | Cohort A    | 3.75% | 1.90% | 4.67% | 1.89% | 2.03% |
|               | Cohort B    | 3.37% | 1.83% | 7.40% | 5.02% | 1.86% |
|               | Cohort C    | 4.94% | 6.90% | 3.58% | 4.82% | 2.86% |

Abbreviations: CV, coefficient of variation; RT, room temperature.

### FIGURE 4 Dilution linearity. Linearity curves of dilution ratios vs actual values of PIVKA-II measured by (A) Lumipulse, (B) Architect was shown. Each line represents one case of data.
patients and demonstrated that 80.50% cases had HBV infection and 80.12% cases had liver cirrhosis.\textsuperscript{33} All these data indicate the importance of HCC surveillance in patients with the context of CHB and cirrhosis in China. In this study, we inquired the optimal cutoff value of PIVKA-II for diagnosing HCC in patients with CHB or cirrhosis. PIVKA-II yielded a ROC curve area of 0.9737, with a sensitivity of 91.78% and a specificity of 96.3%, in discriminating HCC from CHB patients, at a cutoff of 37.5 mAU/mL. PIVKA-II yielded a ROC curve area of 0.9419, with a sensitivity of 77.46% and a specificity of 95.12%, in discriminating HCC from HBV-related cirrhosis patients, at a cutoff at 45 mAU/mL. These values were close to the cutoff value of 40 mAU/mL in the previous reports.\textsuperscript{34} Consistent with other reports, a specificity around 95.12%–96.3% revealed the superior discriminating power of PIVKA-II under the context of CHB and cirrhosis than AFP, supporting the opinion that PIVKA-II is more specific for HCC surveillance in HBV-related high-risk patients.

Although PIVKA-II is regarded as a highly specific HCC biomarker, it is disturbed by vitamin K deficiency or antagonist drugs such as warfarin according to its characteristic as a prothrombin induced by vitamin K absence.\textsuperscript{35} Other research demonstrated that alcoholic liver disease and antibiotics usage could also influence PIVKA-II.\textsuperscript{36} To address this issue, we investigated the distribution of PIVKA-II among chronic liver diseases of different etiologies in this study. Consistent with other literature, alcohol-related liver disease had higher PIVKA-II concentrations regardless of cirrhosis context (ASH vs CHS: 48.80 vs 25.04; AC vs LC: 101.70 vs 55.99). Therefore, high PIVKA-II concentrations in patients with ASH should be interpreted with careful deliberation. In addition, PIVKA-II values in the patients with cirrhosis were comparatively higher than those in the patients with hepatitis (CH vs LC: 25.04 vs 55.99). Elevated PIVKA-II may reflect the progressive liver damage by cirrhosis, and on the other hand, it may be due to the interferences such as obstructive jaundice and cholestasis.\textsuperscript{37} Although the above-mentioned influencing factors were included, 4.81% of 312 patients with chronic hepatitis exceeded PIVKA-II cutoff value (40 mAU/mL), and 12.8% of 289 patients with liver cirrhosis, revealing the satisfactory specificity of PIVKA-II in chronic liver disease.

However, there are still some limitations in this research. First, we concentrated on HCC surveillance in HBV-related live diseases, and thus, we mainly focused on HBV-related patients. Due to the incidence of alcoholic and non-alcoholic fatty liver diseases increases year by year, monitoring of liver cancer in these patients is needed in further research. Second, according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) guidelines for establishing reference intervals (EP28-A3c), 120 observations are the minimum requirements for statistical significance for both parametric and non-parametric data. Due to the time limitation, we did not gather the enough data especially in autoimmune hepatitis and steatohepatitis. Therefore, we investigated the tendency of PIVKA-II distribution among chronic liver disease without statistical analysis.
CONCLUSION

In conclusion, our data indicated that PIVKA-II had satisfactory diagnostic performances in patients with liver diseases of various etiologies and demonstrated that PIVKA-II can be used as a screening or surveillance biomarker in HCC high-risk populations. Furthermore, this research revealed that PIVKA-II was less affected by the patients with HBV-related liver disease, showing its superior diagnostic efficacy than AFP. We believe that the combination of AFP and PIVKA-II will improve the diagnosis and the recurrence detection rate of HCC patients, increase the proportion of early treatment, ultimately improve the survival rate and prolong the life of HCC patients.

ORCID

Jun Ji  https://orcid.org/0000-0002-3278-3594
Chunfang Gao  https://orcid.org/0000-0002-4891-2944

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. 10.3322/caac.21492
2. Ringelhan M, Pfister D, O'Connor T, Pikarsky E, Heikenwalder M. The immunology of hepatocellular carcinoma. Nat Immunol. 2018;19(3):222-232.
3. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. CA Cancer J Clin. 2016;66(2):115-132.
4. Maluccio M, Covey A. Recent progress in understanding, diagnosing, and treating hepatocellular carcinoma. CA Cancer J Clin. 2012;62(6):394-399.
5. Akinyemiju T, Abera S, Ahmed M, et al. The Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional, and National Level: Results From the Global Burden of Disease Study 2015. JAMA Oncol. 2017;3(12):1683-1691.

|    | AFP | PIVKA-II | AFP + PIVKA-II |
|----|-----|----------|----------------|
| Sen (%) | 61.33 | 82.67 | 89.33 |
| Spe (%) | 91.15 | 90.00 | 82.50 |
| AUC (95% CI) | 0.817 (0.773–0.862) | 0.928 (0.906–0.951) | 0.859 (0.825–0.894) |

Note: The diagnostic cutoff values of AFP and PIVKA-II were 20 ng/mL and 40 mAU/mL. The results were collected from 150 HCC patients and 520 disease controls.

FIGURE 5 Distribution of PIVKA-II in chronic liver diseases. Scatter plots of serum levels of PIVKA-II in (A) the chronic hepatitis group and (B) the liver cirrhosis group. The chronic hepatitis group included chronic hepatitis B (CHB), chronic hepatitis C (CHC), alcoholic steatohepatitis (ASH), non-alcoholic steatohepatitis (NASH), and autoimmune hepatitis (AIH) patients. The liver cirrhosis group included HBV-related liver cirrhosis, HCV-related liver cirrhosis, primary biliary cirrhosis (PBC), alcohol-related cirrhosis (AC), and other cirrhosis patients. The number of patients in each group was indicated below, respectively.

FIGURE 6 Optimal cutoff values of PIVKA-II for diagnosing HBV-related HCC. Receiver-operating characteristic curves (ROC) and their corresponding area under the curve (AUC) values for (A) HBV-related HCC patients without cirrhosis (n = 73) vs chronic hepatitis B (n = 81), (B) HBV-related HCC with cirrhosis (n = 71) vs HBV-related liver cirrhosis (n = 82).
6. Polaris OC. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. *Lancet Gastroenterol Hepatol*. 2018;3(6):383–403.

7. Inoue T, Tanaka Y. Hepatitis B virus and its sexually transmitted infection - an update. *Microbial Cell*. 2016;3(9):420–437.

8. Chinese Society of Infectious Diseases CMA, Chinese Society of Hepatology CMA. [The guidelines of prevention and treatment for chronic hepatitis B (2019 version)]. *Zhonghua Gan Zang Bing Za Zhi*. 2019;27(12):938–961.

9. Forner A, Llovet JM, Bruix J. *Hepatocellular carcinoma*. *Lancet*. 2012;379(9822):1245–1255.

10. Malek NP, Schmidt S, Huber P, Manns MP, Greten TF. The diagnosis and treatment of hepatocellular carcinoma. *Deutsches Arzteblatt International*. 2014;111(7):101-106.

11. Waldmann TA, McIntire KR. The use of a radioimmunoassay for alpha-fetoprotein in the diagnosis of malignancy. *Cancer*. 1974;34(58):1510–1515.

12. Liaw YF, Tai DI, Chen TJ, Chu CM, Huang MJ. Alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. *J Hepatol*. 2001;34(4):570–575.

13. Lok AS, Lai CL. *alpha-Fetoprotein monitoring in Chinese patients and treatment of hepatocellular carcinoma*. *Deutsches Arzteblatt International*. 2014;111(7):101-106.

14. Taketa K. Alpha-fetoprotein: reevaluation in hepatology. *Hepatology*. 1990;12(6):1420–1432.

15. Johnson PJ. The role of serum alpha-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. *Clin Liver Dis*. 2001;5(1):110-119.

16. Bruix J, Sherman M. American Association for the Study of Liver Disease. Management of hepatocellular carcinoma: an update. *Hepatology*. 2011;53(3):1020-1022.

17. European Organisation For The Study Of The Liver. Treatment Of C. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol*. 2012;56(4):908-943.

18. Liebman HA, Furie BC, Tong MJ, et al. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. *N Engl J Med*. 1984;310(22):1427–1431.

19. Tanaka Y, Kashiwagi T, Tsutsumi H, et al. Sensitive measurement of serum abnormal prothrombin (PIVKA-II) as a marker of hepatocellular carcinoma. *Hepatogastroenterology*. 1999;46(28):2464-2468.

20. Ikoma J, Kaito M, Ishihara T, et al. Early diagnosis of hepatocellular carcinoma using a sensitive assay for serum des-gamma-carboxy prothrombin: a prospective study. *Hepatogastroenterology*. 2002;49(43):235-238.

21. Carr Bl, Kanke F, Wise M, Satomura S. Clinical evaluation of lens culinaris agglutinin-reactive alpha-fetoprotein and des-gamma-carboxy prothrombin in histologically proven hepatocellular carcinoma in the United States. *Dig Dis Sci*. 2007;52(3):776-782.

22. Marrero JA, Su GL, Wei W, et al. Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in american patients. *Hepatology*. 2003;37(5):1114-1121.

23. Ryu MR, Kang ES, Park HD. Performance evaluation of serum PIVKA-II measurement using HISCL-5000 and a method comparison of HISCL-5000, LUMIPULSE G1200, and ARCHITECT i2000. *J Clin Lab Anal*. 2019;33(6):e22921.

24. Qin X, Tang G, Gao R, et al. A multicenter study on PIVKA reference interval of healthy population and establishment of PIVKA cutoff value for hepatocellular carcinoma diagnosis in China. *Int J Lab Hematol*. 2017;39(4):392-401.

25. Li Y, Chen J. Serum Des-gamma-carboxy Prothrombin for Diagnosis of Adult Primary Cancer in Liver. *J College Phys Surg-Pakistan*. 2019;29(10):972-976.

26. Trevisani F, D’Intino PE, Morselli-Labate AM, et al. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. *J Hepatol*. 2001;34(4):570–575.

27. Yamashita Y, Shirabe K, Aishima S, Maehara Y. Predictors of Microvascular Invasion in Hepatocellular Carcinoma. *Dig Dis*. 2015;33(5):655-660.

28. Ji J, Wang H, Li Y, et al. Diagnostic Evaluation of Des-Gamma-Carboxy Prothrombin versus alpha-Fetoprotein for Hepatitis B Virus-Related Hepatocellular Carcinoma in China: A Large-Scale, Multicentre Study. *PLoS One*. 2016;11(4):e0153227.

29. Xu JF, Liu XY. PIVKA-II is an independent prognostic factor for overall survival of HCC patients and maybe associated with epithelial-mesenchymal transition. *J Hepatol*. 2015;63(4):1040-1041.

30. Chu CM, Liaw YF. Hepatitis B virus-related cirrhosis: natural history and treatment. *Semin Liver Dis*. 2006;26(2):142-152.

31. Hsu YS, Chien RN, Yeh CT, et al. Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. *Hepatology*. 2002;35(6):1522-1527.

32. Fattovich G, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. *J Hepatol*. 2008;48(2):335-352.

33. Zhang B, Zhang B, Zhang Z, et al. 42,573 cases of hepatectomy in chronic liver diseases: A multi-center study in China. *Sci China Life Sci*. 2018;61(6):660-670.

34. Kuromatsu R, Tanaka M, Shimauchi Y, et al. Usefulness of ED036 kit for measuring serum PIVKA-II levels in small hepatocellular carcinoma. *J Gastroenterol*. 1997;32(4):507-512.

35. Umeki S, Umeki Y. Levels of acoxbaprothrombin (PIVKA-II) and coagulation factors in warfarin-treated patients. *Med Lab Sci*. 1990;47(2):103-107.

36. Kang KH, Kim JH, Kang SH, et al. The influence of alcoholic liver disease on serum PIVKA-II levels in patients without hepatocellular carcinoma. *Gut and liver*. 2015;9(2):224-230.

37. Li C, Zhang Z, Zhang P, Liu J. Diagnostic accuracy of des-gamma-carboxy prothrombin versus alpha-fetoprotein for hepatocellular carcinoma: A systematic review. *Hepatol Res*. 2014;44(10):E11-25.

---

**How to cite this article:** Ji J, Liu L, Jiang F, et al. The clinical application of PIVKA-II in hepatocellular carcinoma and chronic liver diseases: A multi-center study in China. *J Clin Lab Anal*. 2021;35:e24013. [https://doi.org/10.1002/jcla.24013](https://doi.org/10.1002/jcla.24013)