Int J Clin Exp Med 2020;13(11):8875-8881
www.ijcem.com /ISSN:1940-5901/IJCEM0118696

Original Article

Diagnostic value of abnormal prothrombin level combined with AFP and AFP-L3% for chronic hepatitis B-related liver cancer

Qiang Wang[1*], Yunqian Xie[2*], Liyuan Zhang[1], Kunliang Wu[1], Yuting Zhu[1], Feiyu Wu[1], Yongguo Du[1]

Departments of [1]Tropical Diseases, [2]Gastroenterology, The Second Affiliated Hospital of Hainan Medical University, Haikou, Hainan Province, China. [*]Equal contributors and co-first authors.

Received July 22, 2020; Accepted August 15, 2020; Epub November 15, 2020; Published November 30, 2020

Abstract: Objective: To investigate the diagnostic value of abnormal prothrombin (PIVKA-II) level combined with alpha-fetoprotein (AFP) and AFP-L3% (AFP/AFP-L3 ratio; AFP-L, alpha-fetoprotein heterogeneity) in patients with chronic hepatitis B-related liver cancer. Methods: A total of 442 patients with chronic hepatitis B (CHB) were selected for this retrospective study, including 144 CHB patients, 167 CHB patients with liver cirrhosis, and 131 CHB patients with liver cancer. Their PIVKA-II AFP and AFP-L3% levels were tested. Results: In CHB patients with liver cancer, the PIVKA-II, AFP and AFP-L3% levels were higher than those of other patients ($P<0.05$). In CHB patients with cirrhosis, the AFP and AFP-L3% levels were higher than in patients who only had CHB ($P<0.05$). In diagnosing CHB related liver cancer, the ROC curve showed that the AUC values of PIVKA-II, AFP and AFP-L3% were 0.855, 0.739, and 0.838, respectively, which were all significantly lower than the PIVKA-II+AFP+AFP-L3% in non cancer (0.987; $P<0.05$). The sensitivity of PIVKA-II, AFP and AFP-L3% were 0.981, 0.798 and 0.663, while the specificity were 0.640, 0.636, 0.981, respectively. If combining both measures for the diagnosis of CHB related liver cancer, the AUC values of PIVKA-II+AFP, PIVKA-II+AFP-L3% and AFP+AFP-L3% were 0.906, 0.958, and 0.841, respectively, which were lower than PIVKA-II+AFP+AFP-L3% alone ($P<0.05$). Conclusion: The combination of PIVKA-II, AFP and AFP-L3% has a high value in the diagnosis of patients with hepatitis B-related liver cancer, and it is worthy of clinical promotion.

Keywords: Hepatitis B, liver cancer, PIVKA-II, alpha-fetoprotein, alpha-fetoprotein heterogeneity, AFP-L3%, diagnostic value

Introduction

Chronic hepatitis B (CHB) is an infectious liver disease caused by a hepatitis B virus infection. China is a country with a high incidence of hepatitis B virus infection. Patients may experience clinical symptoms such as reduced diet, abdominal distension, epigastric pain, and fatigue right after infection [1, 2]. If the control of CHB is poor, the disease can progress further, causing cirrhosis, liver failure, and eventually liver cancer [3, 4]. At present, the incidence of primary liver cancer in China ranks first among all cancers, and it is also one of the most lethal diseases in China [5]. Studies have shown that the main cause of primary liver cancer in China is CHB, thus early diagnosis of hepatitis B-related liver cancer has important clinical significance [6, 7].

Alpha-fetoprotein (AFP) is one of the most common clinical indicators for the diagnosis of liver diseases. However, about 40% of liver cancer patients have a normal AFP level, indicating that the sensitivity of AFP alone is relatively poor [8]. Abnormal prothrombin (PIVKA-II) is a biologically inactive protein synthesized under vitamin K deficiency, which is also a precursor protein of prothrombin. It is mainly synthesized by the liver. Studies have shown that PIVKA-II combined with AFP can improve the diagnosis rate of liver cancer effectively [9]. The heterogeneity of alpha-fetoprotein, AFP-L3, is produced by the combination of AFP and lentil lectin (LCA), which is mainly secreted by liver cancer cells. AFP-L3% is the ratio of AFP and AFP-L3 [10]. Studies have shown AFP-L3% alone had high specificity in diagnosing primary liver cancer [11]. This study explored the diagnosis value

of the combination of PIVKA-II, AFP AFP-L3% for hepatitis B-related liver cancer.

Subjects and methods

General information of patients

This retrospective study collected the data of 442 patients with chronic hepatitis B (CHB) who were admitted to the Second Affiliated Hospital of Hainan Medical University from April 2017 to April 2020, including 144 CHB patients (107 males and 37 females, aged 18-76 years old), 167 CHB patients with cirrhosis (112 males and 55 females, aged 24-77 years old), 131 CHB patients with liver cancer (115 males and 16 females, aged 26-78 years old). All patients signed the informed consent. The study was approved by the Ethics Committee of the Second Affiliated Hospital of Hainan Medical University.

Inclusion and exclusion criteria

Inclusion criteria were as follows: Patients were diagnosed with CHB or CHB combined with cirrhosis according to the Chinese Guidelines for the Prevention and Treatment of Chronic Hepatitis B (2019 Edition) [12]; patients were diagnosed with liver cancer which was confirmed by pathology according to the diagnostic criteria of the Chinese Diagnosis and Treatment of Primary Liver Cancer (2019 Edition) [13]; patients were more than 18 years old; patients with complete clinical data.

Exclusion criteria were as follows: Patient had non-Hepatitis B virus infections such as Hepatitis A, Hepatitis C, and Hepatitis E; patients were taking vitamin K, warfarin, dabigatran or other drugs that might affect coagulation function; patients had other gastrointestinal cancers that might affect AFP levels; patients had liver cancer relapse after resection or metastatic liver cancer.

Methods

Determination of PIVKA-II, AFP and AFP-L3% levels: Two tubes of 5 mL venous blood were collected at the time of admission. The collected blood samples were stored in the refrigerator at 4°C in a sterile tube with ethylenediaminetetraacetic acid. After 15 minutes, the samples were centrifuged at 4,000 rpm for 5 minutes to obtain the serum and plasma, respectively. The plasma was mixed with phosphate buffer solution containing 40 μL of protease inhibitor and then stored in a freezer at -40°C. AFP and PIVKA-II levels were measured using automatic chemiluminescence immunoassay analyzer (model i2000SR, Abbott, United States) according to the instruction of kits purchased from Abbott Trading (Shanghai) Co., Ltd., China. AFP-L3 kit was purchased from Beijing Hotgen Biotech Co., Ltd., China. AFP-L3% = AFP/AFP-L3 * 100. AFP <9 ng/mL was defined as negative, AFP ≥9 ng/mL was defined as positive. PIVKA-II <40 mAU/mL was defined as negative, PIVKA-II ≥40 mAU/mL was defined as positive. AFP-L3 <2.5 mg/L was defined as negative, AFP-L3 ≥2.5 mg/L was defined as positive. AFP-L3% <0.1 was defined as negative, AFP-L3% ≥0.1 was defined as positive.

Outcome measures

The PIVKA-II, AFP and AFP-L3% levels of all patients were measured and compared among groups. The ROC diagnostic curve was used to describe the diagnostic efficacy of PIVKA-II, AFP, AFP-L3% alone, PIVKA-II+AFP, PIVKA-II+AFP-L3%, AFP+AFP-L3%, and PIVKA-II+AFP+AFP-L3% in CHB patients with liver cancer. The area under curve m(AUC) of ROC was also calculated and compared among groups.

The occurrence of liver cancer was set as the dependent variable; a multi-factor Logistic regression model was constructed to analyze related factors.

Statistical analysis

SPSS 17.0 statistical software was used for statistical processing. The measurement data were tested for normality. Data conformed to normal distribution were expressed using mean ± standard deviation ($\bar{x} \pm sd$) and tested using an independent sample t-test. Data that did not conform to a normal distribution were expressed using M (P25, P75) and tested using a rank-sum test. Enumeration data were expressed as case number and percentage and tested with Pearson chi-square (χ^2). ROC diagnostic curve was adopted to evaluate the value of diagnosis, and Medcalc software was used to draw the ROC curve to calculate the area under the curve. Z test was used to compare the differences between different ROC

Table 1. Comparison of general information of three groups of patients

Item	CHB (n=144)	CHB with cirrhosis (n=167)	CHB with liver cancer (n=131)	χ^2/F	P
Age (year)	37.3±6.9*,#	50.6±5.8	53.2±6.7	249.812	<0.001
Gender (male/female)	107*/37	112*/55	115/16	17.245	<0.001
Course of disease (year)	8.73±3.21*	9.79±3.12*	11.23±2.43	24.51	<0.001
Body mass index (kg/m^2)	22.36±2.98	21.98±2.78	21.87±3.02	1.098	0.334
Comorbid disease					
Hypertension (n)	24	32	39	4.573	0.102
Diabetes (n)	20	26	25	1.422	0.491
Hypoproteinemia (n)	10*	42*	85	111.595	<0.001
Stages of liver cancer (I/II/III/IV)			26/58/29/18		
HBeAg positive (n)	37	46	39	0.571	0.752
Quantitative HBeAg (IU/mL)	2562.9±1706.3	2687.6±1798.6	2710.1±1839.4	0.754	0.283

Note: Compared with CHB with liver cancer group, *P<0.05; Compared with CHB with cirrhosis group, #P<0.05. CHB: chronic hepatitis B.

curves. The occurrence of liver cancer was set as the dependent variable and a multi-factor Logistic regression model was constructed to analyze the independent risk factors for the development of liver cancer. P<0.05 indicated that the difference was statistically significant.

Results

Comparison of general information

In this study, the average age of patients with CHB combined cirrhosis group and CHB combined with liver cancer group was higher than that of the CHB group (P<0.05). The proportion of males and disease course in CHB with liver cancer group was higher than that of CHB group and CHB with liver cirrhosis group (P<0.05). The incidence of hypoproteinemia in the CHB with liver cancer group was higher than that in the CHB group and the CHB with liver cirrhosis group (P<0.05). There was no statistical difference in body mass index, HBeAg positive rate, and HBeAg amount (IU/mL) among the three groups (P>0.05). See **Table 1**.

Comparison of PIVKA-II, AFP and AFP-L3% levels

The PIVKA-II, AFP and AFP-L3% levels of CHB with liver cancer group were higher than those of CHB group and CHB with cirrhosis group. The AFP and AFP-L3% levels of CHB with liver cirrhosis group were higher than those of the CHB group (all P<0.05). See **Table 2**.

Comparison of the diagnostic efficacy of the three different indicators

In the diagnosis of liver cancer, the AUC values of PIVKA-II, AFP, and AFP-L3% levels alone were 0.855, 0.739, and 0.838, respectively, which were lower than the combined diagnosis AUC value of PIVKA-II+AFP+AFP-L3% (0.987). After combining three indicators, Logistic regression was used to obtain the best diagnosis model to evaluate liver cancer risk. The equation was Logit(P) = - 5.147+9.452 * PIVKA-II+4.598 * AFP+8.498 * AFP-L3%, The risk probability value (P) refers to the probability in predicting the occurrence of liver cancer based on risk factors, (P) = $e^{-(-5.147+9.452 * \text{PIVKA-II}+4.598 * \text{AFP}+8.498 * \text{AFP-L3\%})}$. The sensitivity of PIVKA-II for the diagnosis of liver cancer is 0.981, the specificity is 0.640. The sensitivity of AFP for the diagnosis of liver cancer is 0.798, the specificity is 0.636. The sensitivity of AFP-L3% for the diagnosis of liver cancer is 0.663, the specificity is 0.981. When combining two different indicators to evaluate liver cancer risk, Logistic regression was also used to obtain the diagnosis model. For PIVKA-II+AFP, the equation was Logit(P) = -4.845+ 8.126 * PIVKA-II+4.269 * AFP; for PIVKA-II+ AFP-L3%, the equation was Logit(P) = -4.695+ 8.024 * PIVKA-II+7.964 * AFP-L3%; for AFP+ AFP-L3%, the equation was Logit(P) = -4.529+ 4.364*AFP+8.012*AFP-L3%. The AUC values of PIVKA-II+AFP, PIVKA-II+AFP-L3% and AFP+ AFP-L3% for the diagnosis of liver cancer were 0.906, 0.958 and 0.841, which were lower than the combined diagnosis AUC value of

Table 2. Comparison of the levels of PIVKA-II, AFP and AFP-L3% in three groups of patients

Group	PIVKA- II (m AU/mL)	AFP (ng/mL)	AFP-L3%
CHB (n=144)	22.82±11.23	3.26 (2.19-15.23)	4.89±1.53
CHB with cirrhosis (n=167)	25.32±8.82	20.13 (3.67-98.89)*	7.07±2.56*
CHB with liver cancer (n=131)	447.23 (57.23, 7293.67)*,#	412.54 (9.27, 2021.76)*,#	14.23±6.23*,#
F	1245.76	789.34	221.21
P	<0.001	<0.001	<0.001

Note: Compared with CHB with liver cancer group, *P<0.05; Compared with CHB with cirrhosis group, #P<0.05. CHB: chronic hepatitis B; AFP: Alpha-fetoproteins.

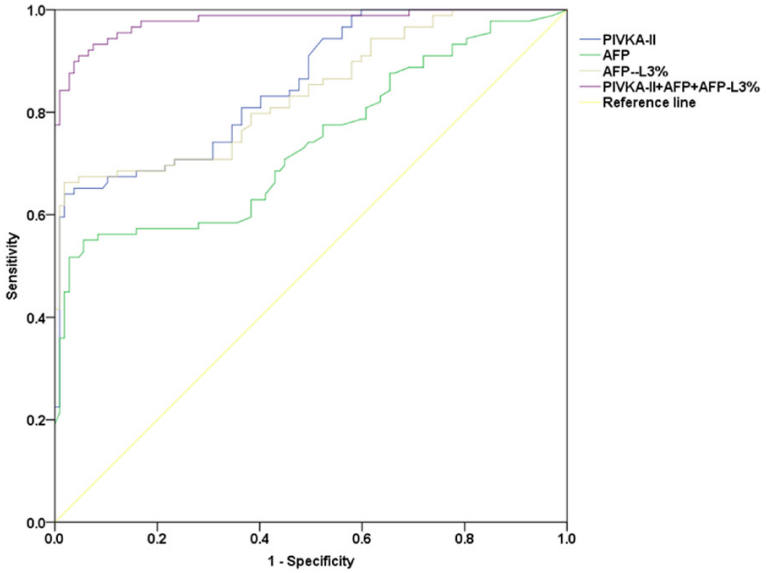

Figure 1. ROC curves of using three separate indicators and all three combined to diagnose liver cancer. AFP: Alpha-fetoprotein; ROC: receiver operating characteristic.

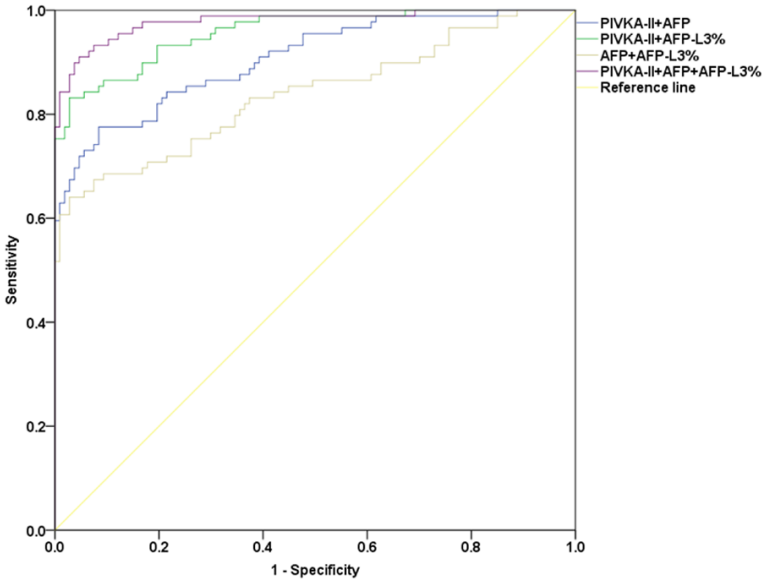

Figure 2. ROC curves of using duo combined indicators and all three combined to diagnose liver cancer. AFP: Alpha-fetoprotein; ROC: receiver operating characteristic.

PIVKA-II+AFP+AFP-L3% (0.987). See **Figures 1**, **2** and **Table 3**.

Multivariate logistic regression analysis of patients

Factors with statistically significant differences in univariate analysis were further screened for multivariate logistic regression analysis. The dependent variable was the occurrence of liver cancer. The independent variables were age, gender, disease course, PIVKA-II, AFP and AFP-L3% levels. Results showed that PIVKA-II, AFP and AFP-L3% levels were independent risk factors for the development of liver cancer (P<0.05). See **Tables 4** and **5**.

Discussion

In 2018, 780,000 people died of hepatocellular carcinoma worldwide and hepatocellular carcinoma is the most lethal cancer in China [5]. Studies showed that the incidence of liver cancer is higher in males and by 2030, the prevalence of primary liver cancer may reach its peak in China [14]. Our study also confirmed that the majority of patients with CHB and liver cancer were male. The male proportion was higher in patients with CHB alone or CHB combined with cirrhosis. The average age of liver cancer patients was older, likely because that

Table 3. AUC and comparison of PIVKA-II, AFP and AFP-L3% in predicting liver cancer

Item	AUC	SD	95% CI
PIVKA-II	0.855	0.026	0.803-0.907
AFP	0.739**	0.031	0.737-0.857
AFP-L3%	0.838##	0.029	0.781-0.895
PIVKA-I+AFP	0.906*,###,&	0.021	0.865-0.948
PIVKA-II+AFP-L3%	0.958**,###,&&,a	0.013	0.932-0.983
AFP+AFP-L3%	0.841##,a,bb	0.030	0.779-0.897
Combined of three indicators	0.978***,###,&&&,aa,b,ccc	0.010	0.960-0.997

Note: Compared with PIVKA-II AUC, *P<0.05, **P<0.01, ***P<0.001; Compared with AFP AUC, ##P<0.01, ###P<0.001; Compared with AFP-L3% AUC, &P<0.05, &&P<0.01, &&&P<0.001; Compared with PIVKA-II+AFP AUC, aP<0.05, aaP<0.01; Compared with PIVKA-II+AFP-L3% AUC, bP<0.05, bbP<0.01; Compared with AFP+AFP-L3% AUC, cccP<0.001. AFP: Alpha-fetoproteins; AUC: area under the curve. CI: confidence interval.

Table 4. Independent variable assignment table of risk factors of liver cancer after hepatitis B infection

Factor	Independent variable	Assignment
Age	X1	≥50 years old =1, <50 years old =0
Gender	X2	Male =1, Female =0
Course of disease	X3	≥10 years =1, <10 years =0
PIVKA-II	X4	Positive =1, Negative =0
AFP	X5	Positive =1, Negative =0
AFP-L3%	X6	Positive =1, Negative =0

Note: AFP: Alpha-fetoprotein.

Table 5. Multivariate logistic regression analysis of patients with liver cancer after hepatitis B infection

Variable	b	S_b	Wald χ^2	*P* Value	95% CI	
					Upper limit	Lower limit
Age	0.702	1.622	1.624	0.156	0.075	47.265
Gender	0.526	0.684	2.698	0.524	0.169	2.068
Course of disease	0.704	1.529	1.614	0.178	0.086	7.598
PIVKA-II	0.765	0.342	9.532	0.000	1.113	4.136
AFP	0.266	0.078	5.108	0.017	1.126	1.532
AFP-L3%	0.495	0.127	8.135	0.003	1.269	2.109

Note: AFP: Alpha-fetoprotein; CI: confidence interval.

liver disease is a slow-progressing process. Since its high morbidity and high mortality, it is of great significance to diagnose and treat liver cancer early.

Early stages of liver cancer often lack clinical symptoms. Most patients were found to be in the middle and late stages of liver cancer when diagnosed, and thus patients have missed the best time to treat cancer and generally have a poor prognosis. The level of AFP in the human body is closely related to the occurrence and development of liver cancer. However, some studies have shown that about 40% of liver cancer patients had normal AFP, and its diagnostic value in liver cancer <3 cm is poor. In those cases, other indicators were often combined to eventually diagnose the disease [15]. Recently, the value of PIVKA-II in the diagnosis of liver cancer has raised clinical attention. Studies have confirmed that patients with AFP-negative liver cancer could use PIVKA-II, which also showed similar efficacy in AFP-positive liver cancer, for cancer diagnosis. The sensitivity of PIVKA-II alone was reported to be 72.8%, the specificity was 88.2%, the AUC was 0.858, which was consistent with our results [16]. Some studies have also shown that PIVKA-II was more sensitive than AFP in the diagnosis of liver cancer [17]. It is generally believed that PIVKA-II can be used with AFP for the diagnosis of liver cancer [18]. Some studies have shown that the diagnostic efficacy of PIVKA-II alone was lower than that of PIVKA-II and AFP combined [19-21]. Other studies have shown that PIVKA-II alone is insufficient for the diagnosis of liver cancer [22, 23].

AFP-L3% is a glycoprotein produced by hepatocytes, studies have shown that AFP-L3% could be used to identify early liver cancer even before the appearance of cancer imaging. It has a high specificity and great value of predicting progression-free survival, thus it is consid-

ered clinically as a new marker of liver cancer [24]. A study adopted the above three indicators to diagnose liver cancer with an AUC of 0.929, which reflected a great diagnostic efficacy in diagnosing liver cancer [25]. However, the relationship between those three indicators and relevant risk factors for the occurrence of liver cancer has not yet been studied. The results of our study showed that PIVKA-II, AFP, and AFP-L3% levels in the CHB with liver cancer group were higher than those in the CHB and CHB with cirrhosis group. It was also found that PIVKA-II had better sensitivity and AFP-L3% had better specificity. Further analysis found that the diagnosis performance of PIVKA-II+AFP-L3% combination was superior to other two combinations. The combination of three different indicators had better diagnosis value than three indicators alone or two of them combined, suggesting PIVKA-II+AFP+AFP-L3%, the combination of three different indicators had good diagnostic efficacy and clinical value. This combination of PIVKA-II, AFP and AFP-L3% could become a complementary role in the diagnosis of liver cancer.

However, there are some limitations of this study. For example, this study had a small sample size, which needs to be further expanded. Besides, this study was a retrospective study, which was subjected to many external factors, such as different treatment options, differences in diagnosis time. There may be selection bias, and further multi-center randomized controlled studies can be conducted.

In summary, PIVKA-II combined with AFP and AFP-L3% showed its high diagnosis value in patients with hepatitis B-related liver cirrhosis and liver cancer, and it is worthy of clinical application.

Disclosure of conflict of interest

None.

Address correspondence to: Yongguo Du, Department of Tropical Diseases, The Second Affiliated Hospital of Hainan Medical University, No. 48 Baishuitang Road, Longhua District, Haikou 570311, Hainan Province, China. Tel: +86-13322066480; E-mail: duyongguo@163.com

References

[1] Rathi A, Kumar V, Majhi J, Jain S, Lal P and Singh S. Assessment of knowledge, attitude, and practices toward prevention of hepatitis B infection among medical students in a high-risk setting of a newly established medical institution. J Lab Physicians 2018; 10: 374-379.

[2] Xu Y and Nie ZW. Telbivudine and adefovir dipivoxil combination therapy improves renal function in patients with chronic hepatitis B: a STROBE-compliant article. Medicine (Baltimore) 2018; 97: e13430.

[3] Wang WT, Zhao XQ, Li GP, Chen YZ, Wang L, Han MF, Li WN, Chen T, Chen G, Xu D, Ning Q and Zhao XP. Immune response pattern varies with the natural history of chronic hepatitis B. World J Gastroenterol 2019; 25: 1950-1963.

[4] Lei JH, Peng F, Chen Z and Xiao XQ. Is HBV viral load at admission associated with development of acute-on-chronic liver failure in patients with acute decompensation of chronic hepatitis B related cirrhosis? BMC Infect Dis 2019; 19: 363.

[5] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and 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: 394-424.

[6] Li T, Qu Y, Wang Y, Lin C, Yang B and Wang L. Entecavir and low genetic barrier antiviral agents for hepatocellular carcinoma in hepatitis b viral cirrhosis: propensity score matching. J Coll Physicians Surg Pak 2019; 29: 317-323.

[7] Liu Z, Jiang Y, Yuan H, Fang Q, Cai N, Suo C, Jin L, Zhang T and Chen X. The trends in incidence of primary liver cancer caused by specific etiologies: results from the Global Burden of Disease Study 2016 and implications for liver cancer prevention. J Hepatol 2019; 70: 674-683.

[8] Wilson SR, Lyshchik A, Piscaglia F, Cosgrove D, Jang HJ, Sirlin C, Dietrich CF, Kim TK, Willmann JK and Kono Y. CEUS LI-RADS: algorithm, implementation, and key differences from CT/MRI. Abdom Radiol (NY) 2018; 43: 127-142.

[9] Zhang D, Liu Z, Yin X, Qi X, Lu B, Liu Y and Hou J. Prognostic value of PIVKA-II in hepatocellular carcinoma patients receiving curative ablation: a systematic review and meta-analysis. Int J Biol Markers 2018; 33: 266-274.

[10] Park SJ, Jang JY, Jeong SW, Cho YK, Lee SH, Kim SG, Cha SW, Kim YS, Cho YD, Kim HS, Kim BS, Park S and Bang HI. Usefulness of AFP, AFP-L3, and PIVKA-II, and their combinations in diagnosing hepatocellular carcinoma. Medicine (Baltimore) 2017; 96: e5811.

[11] Ma H, Sun X, Chen L, Cheng W, Han XX, Zhao B and He C. Multiplex immunochips for high-accuracy detection of AFP-L3% based on surface-enhanced raman scattering: implications for early liver cancer diagnosis. Anal Chem 2017; 89: 8877-8883.

[12] Chinese Society of Infectious Diseases, Chinese Medical Association, Chinese Society of

Hepatology and Chinese Medical Association. Guidelines for the prevention and treatment of chronic hepatitis B (version 2019). J Clin Hepatol 2019; 35: 2648-2669.

[13] Bureau of Medical Administration and National Health Commission of the People's Republic of China. Standardization for diagnosis and treatment of hepatocellular carcinoma (2019 edition). Chin J Dig Surg 2020; 19: 1.

[14] Omata M, Cheng AL, Kokudo N, Kudo M, Lee JM, Jia J, Tateishi R, Han KH, Chawla YK, Shiina S, Jafri W, Payawal DA, Ohki T, Ogasawara S, Chen PJ, Lesmana CRA, Lesmana LA, Gani RA, Obi S, Dokmeci AK and Sarin SK. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. Hepatol Int 2017; 11: 317-370.

[15] Sengupta S and Parikh ND. Biomarker development for hepatocellular carcinoma early detection: current and future perspectives. Hepat Oncol 2017; 4: 111-122.

[16] Chen J, Wu G and Li Y. Evaluation of serum des-gamma-carboxy prothrombin for the diagnosis of hepatitis B virus-related hepatocellularcarcinoma: a meta-analysis. Dis Markers 2018; 2018: 8906023.

[17] Svobodova S, Karlikova M, Topolcan O, Pecen L, Pestova M, Kott O, Treska V, Slouka D and Kucera R. PIVKA-II as a potential new biomarker for hepatocellular carcinoma - a pilot study. In Vivo 2018; 32: 1551-1554.

[18] Huang S, Jiang F, Wang Y, Yu Y, Ren S, Wang X, Yin P and Lou J. Diagnostic performance of tumor markers AFP and PIVKA-II in Chinese hepatocellular carcinoma patients. Tumour Biol 2017; 39: 1010428317705763.

[19] Li Y and Chen J. Serum des-gamma-carboxy prothrombin for diagnosis of adult primary cancer in liver. J Coll Physicians Surg Pak 2019; 29: 972-976.

[20] Li GJ, Chen QY, Harrison TJ, Wang XY, Hu LP, Yang QL, Li KW and Fang ZL. Des-γ carboxyprothrombin may not be a good biomarker for hepatocellular carcinoma in those chronically infected with hepatitis B virus with basal core promoter double mutations (T^{1762}, A^{1764}), a prospective study. Cancer Biomark 2017; 18: 241-248.

[21] Park SJ, Jang JY, Jeong SW, Cho YK, Lee SH, Kim SG, Cha SW, Kim YS, Cho YD, Kim HS, Kim BS, Park S and Bang HI. Usefulness of AFP, AFP-L3, and PIVKA-II, and their combinations in diagnosing hepatocellular carcinoma. Medicine 2017; 96: e5811.

[22] Xing H, Zheng YJ, Han J, Zhang H, Li ZL, Lau WY, Shen F and Yang T. Protein induced by vitamin K absence or antagonist-II versus alpha-fetoprotein in the diagnosis of hepatocellular carcinoma: a systematic review with meta-analysis. Hepatobiliary Pancreat Dis Int 2018; 17: 487-495.

[23] Best J, Bechmann LP, Sowa JP, Sydor S, Dechêne A, Pflanz K, Bedreli S, Schotten C, Geier A, Berg T, Fischer J, Vogel A, Bantel H, Weinmann A, Schattenberg JM, Huber Y, Wege H, von Felden J, Schulze K, Bettinger D, Thimme R, Sinner F, Schütte K, Weiss KH, Toyoda H, Yasuda S, Kumada T, Berhane S, Wichert M, Heider D, Gerken G, Johnson P and Canbay A. GALAD score detects early hepatocellular carcinoma in an international cohort of patients with nonalcoholic steatohepatitis. Clin Gastroenterol Hepatol 2020; 18: 728-735.

[24] Lim TS, Kim DY, Han KH, Kim HS, Shin SH, Jung KS, Kim BK, Kim SU, Park JY and Ahn SH. 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: 344-353.

[25] Hu D, Ma MT, Li MY, Wang FC, Zhang LJ and Sun H. Clinical application of combined detection of protein induced by vitamin K absence or antagonist-II, alpha-fetoprotein and Lens culinaris agglutinin-reactive fraction of AFP in hepatocellular carcinoma. J Shanxi Med Univ 2019; 4: 405-409.